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ELEMENTS OF

VEGETABLE HISTOLOGY

BY

DANIEL BASE

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ELEMENTS OF

VEGETABLE HISTOLOGY

For the Use of Students of Pharmacy, Preparatory
to the Study of Pharmacognosy

WITH 65 ILLUSTRATIONS

BY
DANIEL BASE, Ph.D.,
Professor of Chemistry and Vegetable Histology in the Department of Pharmacy,
University of Maryland, Baltimore

THIRD EDITION, REVISED AND ENLARGED

BALTIMORE, MD.
THE AUTHOR
1912

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DANIEL BASE

PREFACE

This book is intended to serve as a guide for beginners in the study of plant tissues with the microscope, with supplemental instruction from the teacher.

The greater portion of the lessons deals with the tissues and their arrangement in the higher plants, but a few lessons are also included on the simplest plants, namely, some of those of the Thallophyte series, not only on account of their great importance in the life economy, but also to show the gradual increase in complexity of structure in passing from the extremely simple and minute plants of the Thallophyte series to the complicated, highly organized members of the Phanerogams, thus giving the student a view of the structure and characteristics of the whole range of the vegetable kingdom.

The opening pages deal with the physics of light rays in relation to reflection, refraction, lenses, formations of an image, with the description and explanation of the action of a compound microscope, its defects, the requirements for a good instrument, the measurement of magnification and of the size of objects, and the directions for using the microscope. Such information, of course, is necessary to one who pursues a course in plant histology, but it has an additional value that students are apt to overlook.

It enables the pharmacist to listen and speak intelligently when topics arise in which the microscope, etc., are a factor, and he is a better salesman in such places where microscopes and accessories are on sale.

In this day of Pure Food and Drug Laws, Pharmacognosy, or the morphological and histological study of individual official and unofficial plants for the purpose of recognition and detection of adulterations, is of great importance. To carry on such work intelligently requires, as a preliminary preparation, a general knowledge of botany and a working familiarity with general plant histology. This general plant histology the lessons in this book attempt to teach. It is not a book on Pharmacognosy, as it does not attempt to give the details by which one plant organ may be distinguished from another of the same class. For such purpose the student must be referred to works on Pharmacognostic microscopy, of which a goodly number have appeared in late years.

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In the present edition, numerous corrections and additions have been made to the old text, and three new chapters and three cuts have been added. In one of the new chapters, Pleurococcus belonging to the Algæ, and Glœocapsa and Nostoc belonging to the Schizophytes, all low forms of plants, are described. In another, an exercise in making a permanent stained mount of a section is given in detail. In the third, the examination for tubercle bacillus and gonococcus is described, in the belief that it might be turned to practical use by the pharmacist. Some of the additions made to previous chapters are the following: Eye-piece micrometer and measurement of objects; Microsphæra (Lilac mildew) under Moulds; a study of cell division; the work of the leaf, describing the role of the leaf in the life of the plant.

Section A and B of the Appendix have been entirely rewritten. Section A treats of stains, fixing and hardening agents, preserving fluids, clearing and mounting media, etc. Detailed directions for preparing and using stains, and the other agents mentioned are given. Section B treats of cutting sections, and gives exact details for imbedding specimens in paraffin or celloidin, and making permanent stained mounts of sections of such imbedded objects.

The progressive pharmacist is often called upon by physicians and those in charge of laboratories of hospitals for information in regard to stains, etc., and to prepare them, and therefore a description of such agents ought to be a matter of interest to him. In keeping with this idea, a new section, D, has been added to the Appendix, in which the more commonly used stains for animal histology are described, in the hope that this information will be useful.

In preparing the pages of this book, the author consulted a number of books, to which he desires to acknowledge his indebtedness. The books consulted were: *Encyclopædia Britannica;* Ganot's *Physics;* Behrens' *Botanische Mikroskopie;* Bessey's *Botany;* Goodale's *Physiological Botany;* Bastin's *College Botany,* and *Laboratory Exercises;* Bower's *Practical Botany;* Huxley and Martin's *Practical Biology;* Gray's *Lessons in Botany;* Greenish's *Foods and Drugs;* Sedgwick and Wilson's *General Biology;* Strasburger's *Botanisches Practicum,* and *Lehrbuch der Botanik;* Coulter's *Text Book of Botany;* Steven's *Plant Anatomy;* Chamberlain's *Methods in Plant Histology.*

The author is greatly indebted to his colleagues, Chas. C. Plitt, for aid in revising and adding to the text, and H. A. B. Dunning, for advice and assistance in preparing the section on animal stains.

September, 1912.

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ELEMENTS OF
VEGETABLE HISTOLOGY

THE MICROSCOPE

CHAPTER I.

PRELIMINARY CONSIDERATIONS.

Before describing the mechanical and optical parts of a compound microscope, it is essential to know something about the action of the transparent bodies, as prisms, lenses, etc., on light rays—how a lens forms an image of an object, and how the image is magnified, etc.; in other words, a little of the elementary physics of light.

Light travels through homogeneous, transparent media, as air, water, glass, in straight lines, and a very narrow cylinder of light is called a *ray,* or better, a *pencil* of light. Rays are represented in geometric illustrations by straight lines. The fact that light travels in straight paths may easily be shown by admitting a small beam through a hole in a shutter into a darkened room in which dust particles are floating around. The illuminated particles will be seen to lie in a straight line. The formation of sharp shadows by obstacles in the path of light is another evidence that light travels in straight lines. When the rays come from a distant source, as the sun, moon, stars, a distant flame, they are practically parallel, and a beam of such light is spoken of as parallel light or beam.

A convergent beam of light is one in which the rays come together in a point or focus.

A divergent beam of light is one in which the rays emanate from a point or focus.

Mirrors.—When light falls upon bodies, it is in general reflected. If the surface be rough, the rays will be reflected in every conceivable direction, each point of it becoming, as it were, a new source of light. It is for this reason that rough bodies are seen, and also from any position. When the surface of a body is smooth, the light rays falling upon it are reflected in a definite direction according to fixed laws, and such surfaces are called *mirrors.* "Smoothness" is a relative term, but with reference to light rays, it means that there are no unevennesses which are at all comparable in size with the wave-length of the waves of light, which is very small. When the surface is plane, it is called a ***plane mirror,*** and when spherical, it is called a ***spherical mirror.*** These are the commonest forms, and both are used on microscopes for illuminating objects examined.

Plane Mirror.—The manner in which this reflects light is illustrated in Fig. 1. AB is a vertical section of the mirror, CD is a perpendicular to the surface, OD is an incident ray, and the angle ODC is the angle of incidence, DP is the reflected ray and CDP is the angle of reflection. It has been shown experimentally, as well as deduced theoretically from the wave theory of light, that in a plane mirror the reflection follows a fixed law, namely, that the angle of reflection is always equal to the angle of incidence; that is, the angle CDP is always equal to the angle ODC.

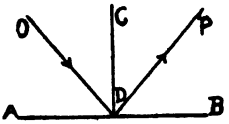


Fig. 1.

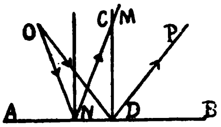


Fig. 2.

What is true for one ray, as illustrated in Fig. 1, is true for any number of rays. Hence if a beam of parallel rays of light be reflected from a plane mirror, the angles of reflection of the rays will all be equal, consequently the reflected beam will consist of parallel rays. Similarly, by geometrical construction based on the above law, it may be shown that divergent rays, after reflection, remain divergent to the same degree as before reflection. This is shown in Fig. 2. It follows, therefore, that the only function of a plane mirror is to change the path of light that falls upon it. For example, on the microscope it causes a beam of light that is received from a window to be thrown up vertically through the object and the lenses.

Spherical Mirror.—As indicated by the name, the reflecting surface of this mirror is part of a sphere, and may be either convex or concave, but only the latter is of interest in connection with the microscope.

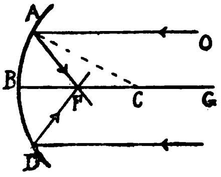


Fig. 3.

In Fig. 3, ABD is a section through the middle point B of the spherical surface, C is the center of curvature. The line GCB through the center C, and the middle point, B, of the mirror, is called the axis. Applying the law of reflection, it may be shown by geometric construction that, in the case of a spherical surface, any ray AO, parallel to the axis BCG, will be reflected to a point F on the axis. This point is called the principal focus and lies half-way between C and B. It will be seen that a beam of light, consisting of rays parallel to the axis, is concentrated or converged to the focus, and hence the illumination at the focus is greatly intensified. If a beam of parallel rays fall upon the mirror at an angle to the axis, it will also be converged to a focus, which, however, does not lie on the axis BCG.

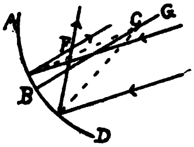


Fig. 4.

Fig. 4 shows the direction of reflection in this case. This is the condition that obtains when the concave spherical mirror is used on a microscope. It has the additional property, as compared with a plane mirror, of concentrating light at the same time that it changes its direction, thus producing a much stronger illumination of objects to be examined.

Refraction.—It was noted above that a light ray travels in a straight line. This is true only when the medium remains the same. Light passing from one medium into a different medium is bent out of its course, still moving, however, in a straight path in the second medium, but in a different direction from that in the first. The bending of light rays is known as *Refraction,* and the action of microscopes depends on this important property.

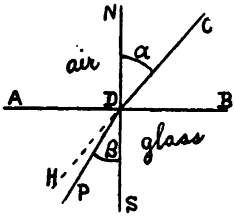


Fig. 5.

Fig. 5 illustrates the principle of refraction. CD is an incident ray of light passing from air into glass at the surface of separation ADB; DP is the refracted ray, NDS is a perpendicular to the surface of the glass, NDC ($=a$) is the angle of incidence, PDS ($=b$) is the angle of refraction, and HDP is the angle through which the ray has been deviated from its original path, CDH. The angle of refraction, b, for a given angle of incidence, a, in air, varies for different media, as glass, water, glycerin, etc., hence the amount of deviation of the refracted ray from the original path is variable for different media. A ray passing from air (which is usually taken as the standard of reference) to a denser medium is always bent toward the perpendicular NDS; that is, the angle of incidence is greater than the angle of refraction. The incident and refracted rays, and the perpendicular to the surface, lie in the same plane. In any medium, for example glass, the size of the angle of refraction varies with the angle of incidence, which means that the direction a ray will take after refraction depends on the slant with which the incident ray meets the refracting medium. There is, however, a constant relation between the angles of incidence and refraction which is expressed by the Law of Refraction. The law is expressed mathematically thus:

$$\frac{\text{sine of angle of incidence}}{\text{sine of angle of refraction}} = \text{n, a constant.}$$

A few illustrations of this law will help to make it clearer. When a ray of light (Fig. 5) passes from air into water at an angle of incidence of 30° ($=a$), the angle of refraction is 22° 5′ ($=b$). From a table of sines, we find

$$\frac{\text{sine } a}{\text{sine } b} = \frac{0.50000}{0.37594} = 1.33.$$

When angle a is 45°, angle b is 32° 7′, and

$$\frac{\text{sine } a}{\text{sine } b} = \frac{0.707107}{0.531659} = 1.33.$$

When a ray passes from air into glass, if $a = 30°$, $b = 19°\ 12'$, and $\frac{\text{sine } a}{\text{sine } b} = \frac{0.500000}{0.328947} = 1.52$. If $a = 45°$, $b = 27°\ 43'$, and

$$\frac{\text{sine } a}{\text{sine } b} = \frac{0.707107}{0.4652} = 1.52.$$

This ratio, n, is called the *index of refraction,* and is different for the various transparent substances, but fixed for each one. It may be accurately determined by physical experiments, and is usually taken with reference to air as standard. There is only one position in which light is not bent out of its path in passing from air into any other medium, namely, when the light rays are perpendicular to the surface of separation of the two media. The index of refraction of water and glass are n = 1.33 water, n = 1.52 glass (crown). Substances for which n is greater than unity are said to be more refracting than air. The refractive index has reference to light of one color only, i. e., to monochromatic light. The yellow light obtained by holding salt in a Bunsen flame is usually employed.

When light passes from a denser medium into air the figure and discussion is just the reverse of the above. A familiar illustration of refraction is shown by dipping a stick obliquely in water, when it will appear bent. The well-known fact that an object under water is not in the place where it seems to be is also explained by refraction.

Refraction by a Prism.—In optics a prism is any transparent medium comprised between two plane faces inclined to each other. The intersection of the two plane faces is called the *edge* and their inclination is called the *refracting angle.* Triangular glass prisms are generally used. Every section perpendicular to the edge is called a *principal section,* which is a triangle in the case of a triangular prism. Fig. 6 represents such a section, A is called the summit and BC the base of the triangle. Let OI be a ray of light falling upon the prism in the plane of the section ABC. It will be refracted twice according to the law of refraction, first as it enters the prism at I, then as it leaves the prism at D, so that the direction of the ray after emerging from the prism will be DS, instead of the original direction OIP. The amount of this deviation depends on the size of the angle of the prism, its material and the angle of incidence of the ray. The angle PRS measures the deviation of the refracted ray from the original path and is called the *angle of deviation.* It is seen that a ray of light is deflected towards the base of the section ABC, hence its source O appears to be elevated or deflected towards the summit A.

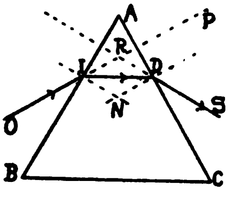


Fig. 6

Light rays of different colors are bent by different amounts, since the refractive indices for the various colors of light are different. White light is a combination of numerous colors, and if a beam of sunlight falls upon a prism it does not come through as white light, but the constituent colors are refracted by different amounts, giving rise to a band of light containing all the colors of the rainbow, namely, red, orange, yellow, green, blue, indigo, violet, red being least refracted, violet most. Such a band of colors is known as a spectrum. An instrument has been constructed for conveniently observing the spectrum of white light which is known as the *Spectroscope.* It has proved to be of the greatest value in chemical analysis. Many new elements were discovered by its aid, for example, calcium, rubidium, thallium, indium, gallium and others.

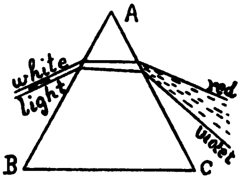


Fig. 7.

The separation of the various colors, due to the unequal refrangibility of the differently colored rays, is known as *dispersion.* We shall speak again of the unequal refrangibility of differently colored rays in connection with lenses and one of their defects, known as chromatic aberration.

Lenses.—A lens is a piece of transparent substance bounded by curved surfaces (one surface may be plane), and, according to the curvature, it may be spherical, cylindrical, elliptical or parabolic. Lenses used in optics are always *spherical* or approximately so. They are usually made either of crown glass, which is free from lead, or of flint glass which contains lead, and is more refractive than crown glass. In virtue of the spherical surfaces, a lens has the property of causing rays of light which traverse it either to converge or diverge. There are six types of lenses according to the manner of combining concave, convex and plane surfaces. These are represented in section in Fig. 8.

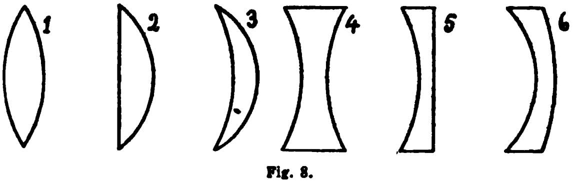


Fig. 8.

They are named, in the order of the numerals, *double convex, plano-convex, converging cancavo-convex, double concave, plano-concave, diverging concavo-convex.*

Lenses 3 and 6 are also called *meniscus lenses,* from the resemblance to the crescent-shaped moon. The first three are thicker in the middle than on the edges and have the power of converging light rays, the last three are thinner in the middle than on the edges and diverge light rays; the first are called *converging* lenses, the latter *diverging* lenses.

For our purpose it will suffice to consider the properties of the two simplest lenses of the series, namely, the biconvex and the biconcave, as the general behavior of the others of the same class is the same.

Biconvex Lens.—Fig. 9 represents a section through the center of a biconvex lens. The faces ADB and ANB are spherical and their centers of curvature are C and C′ respectively,

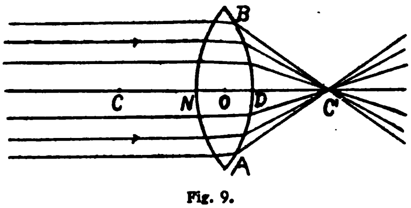


Fig. 9.

which may or may not be equally distant from the center O of the lens. Usually they are equally distant. The line joining C and C′ is called the *optical* or *principal axis.*

If a beam of parallel rays fall upon the lens parallel to the principal axis the rays will be converged to a point called the *principal focus,* which, for a lens of crown glass having surfaces of equal or nearly equal curvatures, coincides very nearly with the center of curvature C′. Of course, there are two focal points, one on each side of the lens, equally distant from the center. This distance C′O is called the *focal length* of the lens, and it varies with the index of refraction of the glass and the radius of curvature of the faces. The shorter the radius of curvature, i. e., the thicker the lens, the shorter is the focal length.

Conversely to the above case, if a divergent beam of rays, emanating from a source placed at the principal focus, fall upon the lens, the rays will emerge parallel to the axis. Fig. 9 illustrates this condition.

If divergent rays emanate from a point on the axis anywhere between the principal focus and a distant point, they will be converged after passing through the lens to a point on the axis, as shown in Fig. 10.

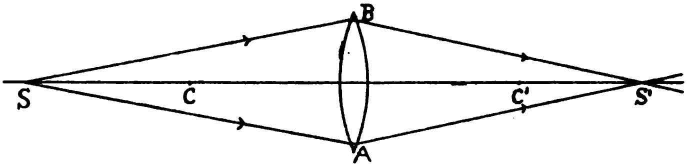


Fig. 10.

There are any number of such related points and they are called *conjugate foci*. If the source of light S be moved away from the lens the focus S′ will approach the lens until it reaches the principal focus C, which happens when S is at a very great distance. On the other hand, if the rays diverge from a point between the principal focus C and the lens, they will still diverge after emerging from the lens, but less so than before, and hence will have no real focal point, but will seem to come from a point S′ behind the lens, as shown in Fig. 11. This apparent focus is known as a *virtual* or *imaginary focus*.

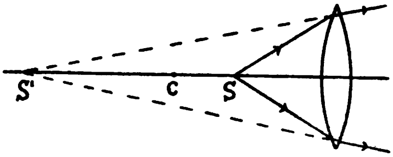


Fig. 11.

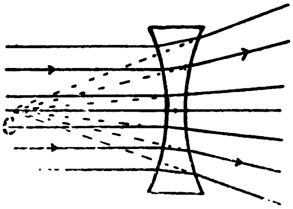


Fig. 12.

Biconcave Lens.—This is just the reverse of the biconvex lens, the spherical surfaces being turned inward, and rays of light diverged or scattered. It has two radii of curvature and a principal axis. Rays of light parallel to the axis are diverged, but if the rays were prolonged backward they would meet in a point or focus, as shown in Fig. 12. The lens has never a real focus, but only an imaginary one. The focus C corresponding to rays parallel to the axis is called the ***principal imaginary focus.***

If rays diverging from any point on the axis fall upon the lens, after emerging they will be still more divergent, and will seem to emanate from a point between the principal focus and the lens. As in the case of a biconvex lens, there are any number of such reciprocally related *conjugate foci.* Fig. 13 represents two such points, S and S′.

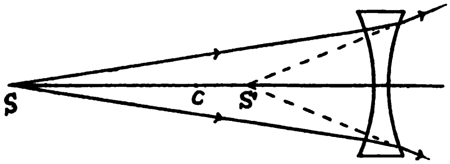


Fig. 13.

The focus S′ approaches the lens more and more as the source of light S is brought nearer to the lens, and vice versa.

Formation of an Image. (1) By double convex lens.—Without describing the geometric construction, the following is the fact: That when a small object is placed near the principal focus but a little distance in front of it, the image formed is at a great distance, is inverted and much larger, and that in proportion as the object is nearer the principal focus. This is shown in Fig. 14, where the arrow A represents a bright body and the arrow B its inverted image, much larger and at a great

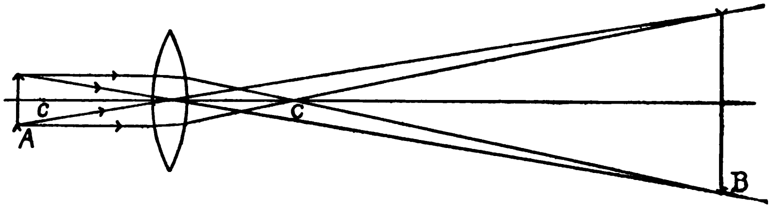


Fig. 14.

distance. C is the focus, the body being a little beyond it. The rays of light coming from every point of A are converged by the lens to a corresponding point in the image B, and the latter is real and can be caught upon a screen held at B. The figure represents what takes place in a compound microscope, as will be shown later. The object and image have the same proportion as their distances from the lens.

When the object is very near the focus, but between it and the lens, the rays from the various points of the object are not converged to a corresponding point as in the previous case, but pass through the lens still diverging, but less so than before, and in such a way that if they were prolonged backward they would meet and form an image behind the object on the same side of the lens as the latter. Fig. 15 will illustrate. An eye held in front of the lens would see the light coming from the object A as if it came from B, and B, therefore, is the image of A, erect, larger, but *unreal* or *imaginary*.

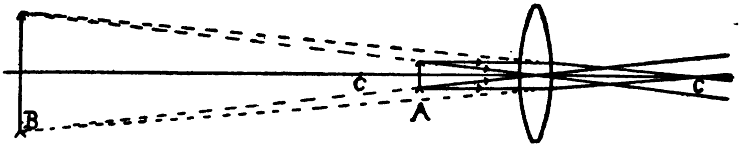


Fig. 15.

A lens used in this manner to form, with the help of the eye, an erect magnified image, constitutes a *simple microscope* or *magnifier*. In both cases considered, the magnification is greater according as the object is nearer the focus and the focal length is decreased, i. e., the lens is more convex. Magnification may also be increased by combining two or three lenses into a "system."

There is a third position which the object may occupy, namely, the principal focus. In this case no image at all is formed, because rays diverging from any point of the object emerge from the lens parallel to one another, and, therefore, have no focus or image. The effects produced by a lens for the three positions described may readily be verified by slowly moving a lens lying on a printed page, vertically upward, when the letters will first appear erect, then vanish and finally return in an inverted position.

(2) By double concave lens.—No real image is ever formed by a concave lens, because it never forms a real focus; it is always virtual, erect, smaller than the object and nearer the

lens than the object. If A be an object in Fig. 16, the rays of light coming from any point of it will emerge from the lens

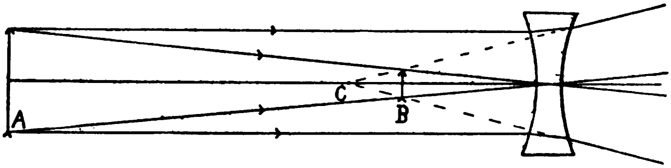


Fig. 16.

more divergent than before, and will appear to the eye to come from a point nearer to the lens and to the axis as shown.

Concave lenses are not used in the microscope except in combination with convex lenses in some objectives.

Spherical and Chromatic Aberration.—These are two serious inherent defects in all simple lenses. They are detrimental to the formation of a perfect image of an object and must be approximately overcome in compound microscopes if these are to be of any value at all.

Cause of Spherical Aberration.—It was said above that parallel rays of light falling upon a double convex lens are converged to a point, but this is not quite true. The rays falling on the edge of the lens are brought to a focus nearer to the lens than those rays falling near the center of the lens, so that the rays, instead of coming together in a point, are focused over a small circle. The diagram (Fig. 17) will illustrate.

The rays around the axis of the lens will meet in a focus C, while those near the edge will meet in C′, and a screen placed at C will not receive a mere point of light, as would be the case if the lens were perfect, but a small circle of light. The result of this is that the image of any object is not sharply defined, but is somewhat blurred in such a manner that when the center of the image is sharp the edge is indistinct and when the edges are sharp the center is indistinct. This defect is due to the spherical nature of the lens, hence its name. As the edge rays are most effective in causing this aberration, the latter can be greatly corrected by cutting out the edge rays by means of a *diaphragm* or perforated disc placed in front of the lens. This is done in objectives and eye pieces of compound microscopes.

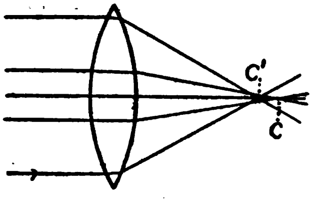


Fig. 17.

Mathematical calculation has shown that spherical aberration is greatly reduced when the radii of curvature of a lens bear a certain ratio to each other, namely, 6:1, the face with longer radius being turned towards the object. Aberration is also corrected in part by combining several lenses of suitable curvatures into a system, the lens next the object being plano-convex, with the plane face towards the object. Absolute correction for spherical aberration is impossible.

Cause of Chromatic Aberration.—We have seen that rays of different colors have different indices of refracton, i. e., unequal refrangibilities, so that if white light is passed through a prism the constituent colors are separated by it into a spectrum. A lens acts like a prism in this respect; in fact, it may roughly be considered as two prisms with their bases together. There is a different focus for each of the seven different colors composing white light; violet being most refracted, is focused nearest the lens, while red being least refracted, is focused farthest from the lens (Fig. 18). The red rays will meet at R, the violet ones at V, and the other colors at points intermediate, in the order, orange, yellow, green, blue, indigo. The result of this defect is that the image of an object is bordered by a color fringe instead of being perfectly colorless, as it should be. Chromatic aberration is more perceptible in proportion as the lenses are more convex, i. e., as the magnifying power increases. It is corrected by combining lenses made from crown and flint glass. The refractive indices of these are very nearly the same, being 1.751 for flint and 1.53 for crown, but the power to separate the colors of white light is nearly twice as great for flint as for crown glass. Hence a biconcave or plano-concave flint glass may be so combined with a biconvex crown glass that the dispersion of one is corrected or compensated by that of the other, while the two still act like a double convex lens in magnifying the object (Fig. 19). Chromatic aberration cannot be corrected absolutely; there will always be a little color, but it may be so little that the image is practically colorless. For optical purposes the blue and orange are corrected or combined. If the image is bordered by a light blue fringe, the lens is said to be *overcorrected;* if by a reddish one, it is *undercorrected.*

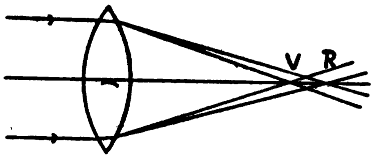


Fig. 18.

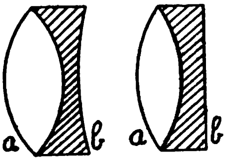


Fig. 19.
a—crown lens.
b—flint lens.

A lens free from chromatc aberration is called *achromatic,* and one free from both spherical and chromatic aberrations is called *aplanatic.*

Simple Microscope.—This is nothing but a convex lens used as a magnifier as described under double convex lens. There may be one lens or several combined into a system and mounted in a suitable stand. Corrected lenses and diaphragms may be used to get rid of spherical and chromatic aberrations. A good example of a simple microscope is a reading glass or a watchmaker's magnifier.

Condition of Distinctness of the Image.—There is for each person a *distance of most distinct vision,* a distance at which an object must be placed before the eye to be seen with greatest distinctness. This distance is, for the average eye, between 12 and 14 inches. It differs for different observers, and the two extremes are found in near and far-sighted persons. When an object is looked at through a lens the latter must be moved back and forth until the image is formed at the particular observer's distance of distinct vision, and this operation is called *focusing.* This explains why two persons looking through a microscope will have quite different foci, since the two eyes have different distances of distinct vision.

The human eye is, in one respect, an optical instrument, because it consists of a combination of lenses which focus upon the retina light rays coming from illuminated objects. The eye has but little spherical aberration, owing to its peculiar shape and to the action of the iris, which takes the place of a diaphragm; but it does have considerable chromatic aberration. Most eyes have power of *accommodation;* that is, of altering their focal length at will so as to perceive objects at different distances away. There are, however, several possible optical defects in eyes, which may arise from various causes:

1. The rays may be brought to a focus in front of the retina instead of on it. Such eyes are called *near-sighted,* and may be helped by the use of diverging lenses which cause the rays to become less divergent in the eye and thus to meet in a focus farther back on the retina.

2. The rays may be focused back of the retina. Such eyes are called far-sighted, and may be helped by the use of converging lenses, which act in a manner opposite to that stated for diverging lenses in 1.

3. The focus may be different for different sections of the eye. If the dial of a clock be looked at an eye may see the figures 2 and 8 clearly, but may not see the 5 and 11 sharply. Such eyes are called *astigmatic,* and may be helped by the use of cylindrical lenses. (Ames' Theory of Physics.)

Measure of Magnification in a Simple Microscope.—The apparent magnitude of an object is the angle it subtends at the eye of the observer. In the case of two objects seen at the same distance, the ratio of the apparent diameters is the same as that of their absolute magnitude. Hence, in a simple microscope (also in a compound one), the magnification is equal to the ratio of the apparent diameter of the image to that of the object, both being at the distance of most distinct vision. But as the apparent diameters are not easy to measure, a simpler method is used which gives an approximate measurement (Fig. 20). AB is an object and A'B' its image, formed at the distance of distinct vision for the eye E. Since the eye is always very close to the lens, the angles subtended by the object and image may be taken as A'OB' and aOb, and the magnification $= \frac{A'OB'}{aOb}$.

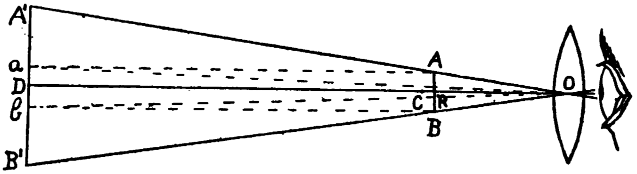


Fig. 20.

This is approximately equal to $\frac{A'B}{ab} = \frac{A'B'}{AB}$, and by similar triangles,

$$\frac{A'B'}{AB} = \frac{\text{DO (dist. of distinct vision)}}{\text{RO (dist. of object from lens)}} = \frac{\text{12 to 14 inches}}{\text{CO (focal length)}},$$

since the object is very nearly at the focus. Hence, magnification by convex lens = ratio of distance of distinct vision (say 13 inches as average) to the focal length of the lens. It will be seen that magnification is greater as the focal length is smaller and as the observer's distance of distinct vision is greater.

COMPOUND MICROSCOPE

The simplest form would consist of two simple microscopes or magnifiers, one with short focus, placed near the object, called *the objective,* the other with longer focus, placed next the eye, and called *the eye-piece* or power. The objective forms an inverted real image of the object, and, by means of the eye-piece, we see a virtual, erect, magnified image of the real image.

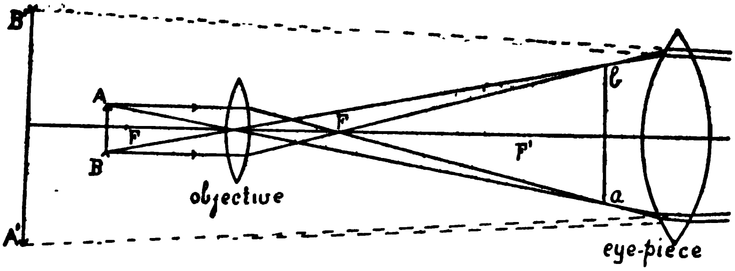


Fig. 21.

Mode of Action.—In Fig. 21, AB is an object; an image is formed at ab, real, inverted and magnfiied. The eye-piece forms an imaginary, erect, magnified image of ab at A'B'. This is the principle of all compound microscopes. This form would be very defective on account of spherical and chromatic aberrations, and we will now study the more perfect microscope.

Description of Compound Microscope.—Fig. 22, A is the base; B, pillar; C, pillar and arm; D, body; E, nose-piece; F, objective; G, ocular; H, draw-tube; I, collar; J, rack and pinion; K, coarse adjustment; L, fine adjustment; N, spring clips; O, mirror; P, mirror bar; Q, diaphragm and substage; R, substage screw; S, stage; T, pillar hinge-joint.

Only a few words need be said about the mechanical parts of the instrument, as the figure will explain sufficiently.

By the rack and pinion movement K, the body D is given a large up-and-down motion and a body is quickly brought into rough focus. Then by the micrometer screw L the fine adjustment is made, a very small motion of the body D being produced by one turn of the screw.

The draw tube H carries a scale so that any tube length can be obtained by pulling out or pushing in. The ocular G slips into the end of the tube H. The triple nose-piece E is a convenience for sliding one or the other objective into place as desired. The stage is perforated in the center for transmitting light, reflected up by the mirror O. In the opening there may be fitted little cylinders with smaller openings, known as diaphragms, the object of which is to regulate the amount of light. There is a series of three or four of these. On the stage are two clips for holding a glass slide, on which the object is examined.

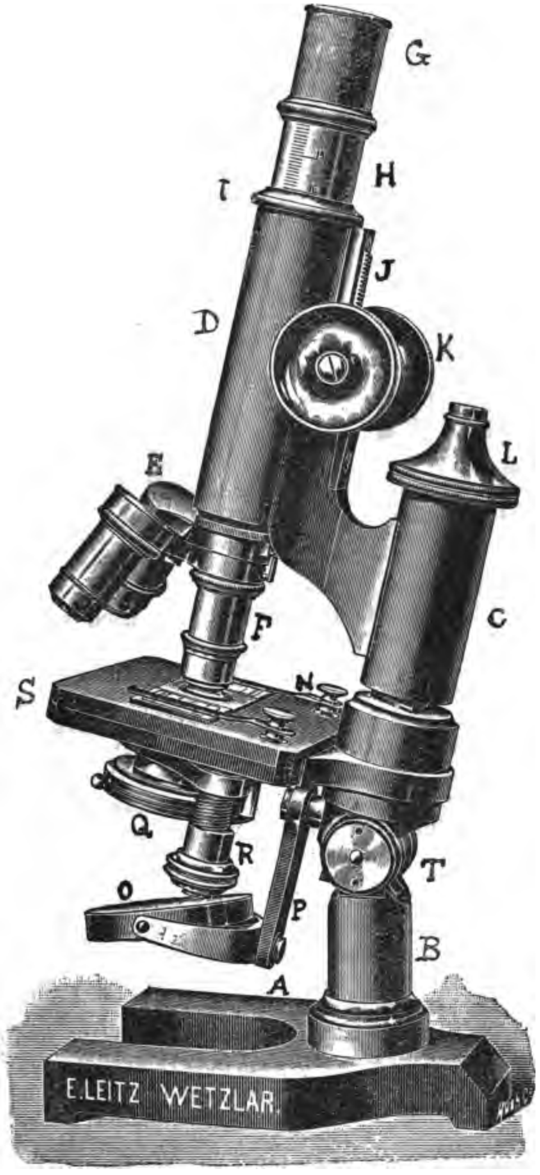
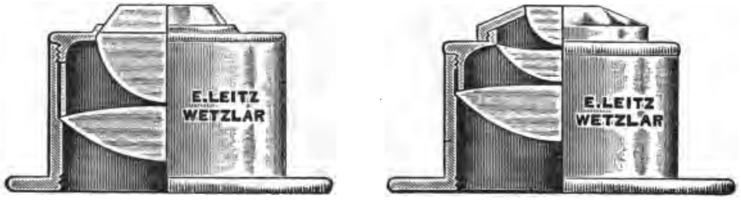


Fig. 22.—Compound Microscope.

The iris diaphragm Q is much more convenient than the cylinder diaphragms, as the opening can be made gradually larger or smaller by simply turning a small lever back or forth.

If a greater concentration of light is desired than is produced by the concave mirror O, a condenser is used, which is placed in position beneath the stage. The best form is the *Abbe type* (Fig. 23), consisting of one lens or a system of lenses for converging a large beam of light. The condenser is used for great magnification and is invaluable in studying stained specimens, which are to be differentiated by color rather than by outline.



Condenser of 1.20 num. apert. Condenser of 1.40 num. apert.

Fig. 23.

Illumination.—No fixed rule can be laid down in regard to the size of opening in the diaphragm to be used for any given magnification, as the amount of light to be passed through a specimen depends somewhat on its nature and thickness. As a general rule, large diaphragms are used for low powers, with weaker illumination and small ones for high powers with strong illumination. Weak illumination is brought about by the plane mirror, stronger by the concave mirror and the use of a condenser if desirable. Actual laboratory practice is better than many words in teaching the student what is the best illumination of an object.

Objectives.—The objectives are the most important parts of the whole microscope. Instead of one lens they consist of a system of two, three or four lenses, some of which are simple, others compounded of a convex crown lens and a concave flint lens, as described under chromatic aberration. The front lens of the system always has a plane face which is turned towards the object, and is usually a simple lens (plano-convex). Such a system of lenses is almost free from aberration defects. According to the method adopted by the maker, objectives are designated by letters, as A, B, C, etc., or by numbers, as 1, 2, 3, etc., or by figures which represent focal lengths. In the latter method, which is the most rational, if an objective is marked, say 1 inch or $^2/_8$ inch, this means that its magnifying power is the same as that of a simple lens whose focal length is 1 inch or $^2/_8$ inch. At present it is customary to express focal lengths of lenses in millimeters instead of inches or fractions thereof. In order to know which is high or low power, the student should remember this rule: *The smaller the number or fraction representing the focal length of an objective, the greater is its magnifying power.* The same rule applies to oculars or eye-pieces. The objectives mostly used in vegetable histology are those of 1 inch, $^2/_8$ inch and $^1/_6$ inch focal length. The distance between the front lens of the objective and the object when in focus is about equal to the focal length of the objective, and is known as the *working distance.*

Objectives are either *dry* lenses or *immersion* lenses. If, as is usually the case, there is an air space between the objective and the object, the lens is called a *dry* one; if a liquid is between the object and the lens, the lens is called an *immersion lens.* The liquid may be water or an oil. If water, we have a *water immersion* lens; if oil, an *oil immersion* lens. If the index of refraction of the oil is about the same as that of glass, we have a *homogeneous immersion* lens. Cedar oil thickened by evaporation is an example of such. Lenses intended for immersion must be constructed accordingly. The great advantage of immersion is that the angle of the cone of light that can be utilized by the lens is considerably increased, thereby increasing the illumination and the efficiency of the microscope.

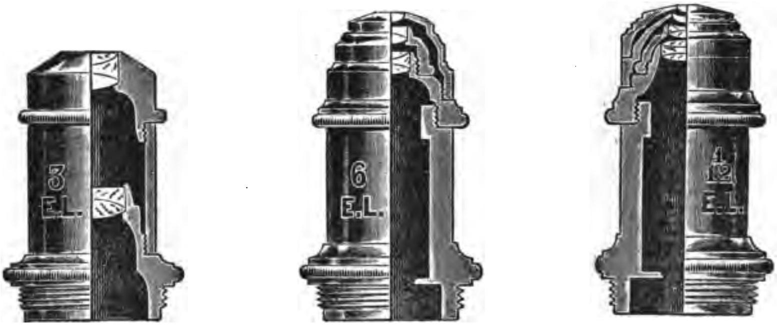


Fig. 24.—Objectives.

Fig. 24 represents the construction of two dry objectives (3 and 6) and an oil immersion objective ($^1/_{12}$ inch focal length). Piece 3 has ¾ inch focal length and consists of two compound lenses or doublets, in which concave and convex lenses are combined. Piece 6 has one single lens and two doublets and a focal length of $^1/_6$ inch. The $^1/_{12}$ inch objective has two single lenses and two doublets. The compound lenses in the pieces serve the purpose of correcting aberration defects. The objectives are achromatic.

Angular Aperture of a Lens.—The efficiency of an objective is in great part dependent upon the size of the cone of light it can take in from a point of the object to form its image. The cone of light utilized is approximately measured by the so-called angular aperture. For a single lens this is the angle formed by lines joining the focal point with the edges of the lens. In an objective it is the angle formed by the lines from the focal point to the edges of the uppermost lens of the system. More accurately, the cone of light utilized is measured by the product of the index of refraction of the medium between the objective and the cover-glass lying over the object, and the sine of half the angular aperture, which is expressed thus, n. sine u, where n is the index of refraction and u is half the angular aperture. This expression is known as the *numerical aperture*. In the case of dry objectives n is 1 (index of refraction of air); for water-immersion objectives n is 1.33; for cedar-oil immersion objectives n is 1.52. It will be seen that the cone of light that can be utilized by an objective of a given angular aperture is considerably greater for an immersion lens than for a dry lens. As the angular aperture varies inversely as the focal length, it follows that the shorter the focal length is, the greater is the cone of light that the lens can utilize; in other words, lenses of high magnification can utilize a greater cone of light than those of low magnification.

Oculars or Eye-Pieces.—The Huyghens' eye-piece is universally used. It is known as a *negative eye-piece*. Its construction is shown in Fig. 25, which consists of two plano-convex crown lenses, the lower one being the larger, less magnifying (its focal length being three times that of the upper lens). It is known as the *field lens*. It increases the *field of vision*, i. e., the number of points of the object that are made visible through the instrument. The upper lens is known as the *eye lens*. It magnifies the image formed by the objective. Both lenses have their convex surfaces turned towards the object. Midway between them is a perforated diaphragm, the object of which is to cut out edge rays from the image and thus decrease spherical aberration. The virtues of the Huyghens' eye-piece are that it corrects chromatic aberration, enlarges the field of vision and forms a flat image, i. e., all points of the image are in focus at the same time. This latter quality is essential in all good microscopes. In all negative eye-pieces the image of the object is formed between the two lenses, and is then further magnified by the *eye lens*. The lenses taking part in the formation of the first image are, there-

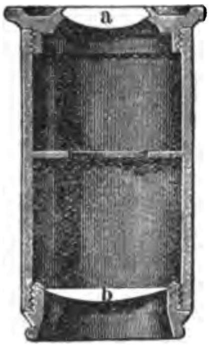


Fig 25.—Huyghens' Eye-Piece.

fore, the objective and the field lens of the eye-piece. In *positive eye-pieces* the first image is formed below the field lens, i. e., the ocular takes no part in its formation. An example of such is Ramsden's eye-piece.

Eye-pieces are designated by methods like those stated for objectives. Those most commonly used have focal lengths of 2 inches, 1½ inches and 1 inch.

There is a particular order that should be observed in changing the lenses in passing from a low power to a high one. For example, there are two objectives, ⅔ and ⅛ inch, and two eye-pieces, 2 and 1 inch. The following is the best order for changing these:

Objective.	Eye-piece.
2/8 inch	2 inch—Low power.
1/8 inch	2 inch—Medium power.
1/8 inch	1 inch—High power.

In other words, it is better to increase magnification at the objective end than at the eye-piece end of the microscope. The reason for this is that the eye-piece magnifies any defects of the objective. In good instruments a high objective is not likely to have more defects than a low one, hence in increasing magnification by changing to a high objective the image will have no more defects than before, although much more magnified.

As the magnifying power is increased the field of view becomes smaller, illumination of the image decreases and the image is increased in size.

Tube Length.—Magnification may also be increased by drawing out the inner tube, which increases the distance between the objective and the plane of the real image formed in the barrel of the instrument, and, consequently, the size of the image. The tube length, however, should be kept constant, because the objectives are prepared to suit a definite tube length, which is sometimes fixed at 160 mm. (6.3 inches), sometimes at 216 mm. (8½ inches). The scale on the inner tube regulates the length.

Camera Lucida.—This is a drawing apparatus which is attached to the eye-piece, and is used whenever it is desired to make accurate delineations of the object. By means of it, a white surface of paper, on the table alongside of the instrument, is reflected into the eye while it receives the image, and thus a pencil point can be traced on the paper along the lines of the image, giving an accurate drawing. The Abbe camera is the best form in the market at the present time. In drawing, the microscope must be erect and the paper horizontal and at the distance of distinct vision, about 12 inches.

Determination of Magnification in a Microscope.—The magnifying power for certain combinations of objectives and eye-pieces and tube length is usually stated by the makers, so that it is hardly necessary now to determine the magnifying power. But sometimes the rating of the makers is not correct and we might want to use a different tube length, and, again, the distance of most distinct vision for our eyes might not be the average distance, namely, 12 to 14 inches, in which case a new determination must be made, which may be done in the following manner:

A Stage Micrometer—a piece of glass, accurately ruled to hundredths of a millimeter—is placed on the stage and brought into focus. By means of a camera lucida, the magnified scale and an accurate mm. scale, placed at the distance of distinct vision alongside the microscope and parallel with the micrometer scale, are brought into superposition. The number of mm. divisions covered by a definite number of the micrometer scale divisions is then noted. Suppose each magnified scale division covers 5 mm. of the rule, what is the magnifying power? One mm. is equal to 100 micrometer divisions, 5 mm. covered by one micrometer space are equal to 500 micrometer divisions. Hence one micrometer division has been magnified so as to cover a space 500 times as wide, i. e., it has been magnified 500 times. This, then, is the power of the instrument for the particular combination.

Eye-Piece Micrometer. Measurement of Microscopic Objects.—For measuring microscopic objects an eye-piece micrometer is essential. This may consist of a small circle of glass, capable of being placed upon the perforated diaphragm of the eye-piece, and upon which is an accurately ruled scale (any scale will do, but one ruled in tenths of a millimeter is preferred). When properly placed the scale should be in focus and lie in the same field of vision as the object to be measured. The number of divisions of the scale covered by the object is then observed. Since the value of each division varies with the objective in use, it is necessary to calibrate the eye-piece micrometer for each objective and tube length that may be used. This is readily done by focusing carefully on a stage micrometer and observing how many divisions of its scale correspond to one division of the eye-piece micrometer. For example, if with a $^{1}/_{6}$ inch objective and 160 mm. tube length 3 of the $^{1}/_{100}$ mm. divisions on the stage micrometer appear to coincide with 10 divisions on the eye-piece micrometer, then 1 division on the latter corresponds to $^{3}/_{100}$ x $^{1}/_{10} = {}^{3}/_{1000}$ mm., or 3 microns. An object observed with this combination and covering 4 divisions of the eye-piece micrometer, measures $4 \mathrm{x} 0.003 = 0.012$ mm., or 12 microns.

The unit for microscopic measurements is the micro-millimeter or micron, which is 0.001 mm. and is represented by the Greek letter μ (English m). Thus if an object measures 15 μ it is 0.015 mm. long.

Source of Light.—The best source of light is a white cloud or the diffused light reflected from a white wall or other white object. *Never* use direct sunlight. Light from the blue sky is not so good as that from a white surface. There is a tendency among beginners to use the strongest light possible. This is injurious to the eyes and often obscures details of the object by its dazzling glare. A window facing north is best.

Requisites of a Good Microscope.—It goes without saying that the best workmanship must be found in the mechanical parts. The foot, pillar, arm, stage, etc., must be of sufficient weight and strength and size. For the optical parts five points must be considered:

1. **Working Distance.**—This is the distance between the front lens of the objective and the object. The lower the magnifying power the larger the working distance in general. Working distance has no fixed relation to the focal length, but varies with the mode of construction and the aperture of the objective. Of two objectives having the same focal length, that one with the larger working distance is to be chosen. As the power is increased the working distance is decreased. It is often advantageous to gain working distance at the expense of magnification as the manipulation of objects on one section slide is made easier.

2. **Penetrating Power or Focal Depth.**—This is the vertical range through which the parts of an object not precisely in the focal plane may be seen with sufficient distinctness to enable their relations with what lies exactly in that plane to be clearly traced out. It is larger the smaller the magnifying power and numerical aperture are, and vice versa. Of two objectives having the same power, but different working distances, that one will have the more focal depth whose working distance is the greater. It is often desirable to see for a considerable distance into an object. In such cases low power must be used.

3. **Flatness of Field.**—All parts of the image must be in focus at the same time.

4. **Defining Power.**—The power to form an image in the highest degree sharply defined, and free from color. This quality is governed by the objectives only and depends on accurate centering of the lenses and completeness of correction for spherical and chromatic aberrations. Want of defining power is indicated by blurring of clearly-marked lines or edges and by general fog.

5. **Resolving Power.**—By which very minute and closely approximated markings, whether lines, striæ, dots or apertures, can be separately discerned. This power varies directly as the aperture of the objective. High powers have the greatest resolving power.

Care of the Microscope.—The stand should *never* be wetted with such substances as alcohol, soap, etc., which dissolve lacquer. If it be necessary to clean the stand, moisten with water and dry with an old linen rag, rubbing with the grain of the brass. Never examine objects lying in acids or alkalies or other chemicals without putting on a "cover" glass. If liquid happens to get on the objective, rinse off at once with water and dry with an old linen rag or Japanese filter paper.

Be careful not to force the lens down on the cover glass. Exercise great care in putting objectives and eye-pieces on or off, lest they be dropped and injured.

DIRECTIONS FOR USING THE MICROSCOPE.

1. The instrument should be placed directly in front of the observer, with the pillar facing backward. Wipe the mirror with a soft rag and turn it so that a beam of light is thrown up through the diaphragm. *All work should be begun with the low-power objective.* The body of the microscope should be about vertical, so as not to interfere with mounting in fluid media.

2. The object mounted on a glass "slide" in a suitable liquid and covered with a "cover glass" is brought to the center of the diaphragm and focused by means of the coarse adjustment in the following manner: Using the left thumb and forefinger to adjust the slide, with the right hand the objective is brought down so that it all but touches the cover glass, then, while looking through the eye-piece, slowly raise the tube by the coarse adjustment until the object is in view; from this point the exact focus can be made by turning the fine adjustment screw.

3. Never lift the slide from the stage, but, having raised the objective, especially in case of high powers, slide it off the stage without upward movement.

4. Accustom yourself to use both eyes indifferently and always keep *both* eyes open. It is preferable to observe with the left eye, as it is more convenient in making drawings.

5. To mount an object, place it in the center of a slide in a drop of liquid, say water; rest a cover glass on its edge near the object in a slanting position and gradually lower it by means of a teasing needle or forceps, in order to avoid entrapping air bubbles. The cover glass should be previously cleaned with a

soft rag or lens paper and then handled by the forceps only. Any superfluous water on the slide is taken up by a camel's-hair brush or blotting paper.

6. Cleanliness should characterize all the work of the microscopical laboratory. All apparatus, slides, cover glasses, etc., should be kept scrupulously free from dirt. The glasses of the objectives and eye-pieces should never be touched with the fingers. Whenever they need cleaning, breathe upon them and wipe with a soft, clean linen rag or a piece of Japanese filter paper.

7. All objects observed should be drawn. Drawings are useful, not only in explaining to others the structures observed, but they are themselves great aids also to accurate observation, and are equally helpful in giving vividness and permanency to knowledge.

Each student should provide himself with a drawing book and a medium pencil. It is excellent practice to keep a record in writing of work done in the laboratory, besides making drawings.

SOME ACCESSORY APPARATUS NECESSARY IN HISTOLOGICAL WORK.

1. Stage micrometer, preferably metric scale. Convenient scale is hundredths of a millimeter.
2. Eye-piece micrometer.
3. Section razor, flat on one side, slightly hollow on the other, for making thin "sections" or slices of bodies; also a hone and a strop.
4. A graduated ruler, having both English and metric scale.
5. Dissecting needles.
6. Sharp-pointed scissors, preferably bent.
7. Delicate forceps or pincettes.
8. Watch glasses for holding sections.
9. Small porcelain evaporating dish.
10. Camel's-hair brushes, assorted sizes.
11. Glass section slides, 3"x1", not too thick, ground edges.
12. Cover glasses, ¾" circles No. 2.
13. Camera lucida for drawing.
14. Polariscope.
15. Draughtsman's dividers, for drawing.
16. Microtome, for section cutting.
17. Turn-table for ringing sections.
18. Pipettes, glass rods, blotting paper.

Microscopical apparatus may be obtained from any large dealer, as Bausch & Lomb Optical Co., Rochester, N. Y.; Queen & Co., Philadelphia. Cuts of apparatus may be seen in catalogues or in larger works on Microscopy, as Behrens' Botanical Microscopy, Gage's The Microscope, etc.

VEGETABLE HISTOLOGY

CHAPTER II.

LINEN, COTTON, SILK, WOOL.

In order to acquire some familiarity with the manipulation of the microscope before studying vegetable objects, it is well to study some simple things like cotton, silk, wool and linen fibres, and these are chosen because they sometimes occur accidentally on the slide when we are studying other things, and also because of their great practical importance.

Linen.—Scrape a linen thread on a glass slide with a knife blade to a woolly mass, mount a *little* of this on a slide in a drop of water, taking care that the fibres are wetted and no air adheres to them, then cover with a cover glass as described on page 29 (5). *The student should guard against an error that beginners are apt to fall into, namely, putting too much material on the slide.* A very small quantity will suffice. Examine with low power ($^2/_3$″ objective and 2″ eye-piece). Very little will be made out. Some clear, smooth, tangled threads will be seen.

Put on high power. The linen will be seen to consist of long, cylindrical fibres, thickened at intervals into nodes, with a small canal looking like a line running lengthwise of the fibre. At intervals there are faint cross-lines (Fig. 26, B). The walls are faintly striated and rather thick, and the canal, which is uniform in width, may contain granular remains of protoplasm. The faint cross-lines become more prominent when the fibres are mounted in a concentrated aqueous solution of chloral hydrate.

Linen fibres are of vegetable origin, their material is *cellulose,* a substance which is one of the chief materials found in plants. Remove the cover glass, add a drop of a solution of iodine in potassium iodide (see reagents), after a few minutes render the fibres nearly dry by removing the liquid with filter paper, then add a few drops of sulphuric acid (see reagents), replace the cover glass and examine again. The fibres are stained a deep blue color and swollen. This is a characteristic test for cellulose material. Iodine alone does not color it, but the acid acts on it, converting it into a starch-like body called *amyloid,* which stains just like starch itself with iodine. Cellulose and starch belong to the same group of chemical compounds, known as *carbohydrates*.

Mount some of the fibres in ammonio-copper hydroxide solution (see reagents) and note that they swell and dissolve quickly, except a slender thread from the center (contents of the canal). The reagent is one of the few solvents for cellulose.

Linen fibres are the so-called *bast fibres* found in the inner bark of the stem of the Flax plant, Linum usitatissimum.

Cotton.—Mount a little raw cotton or non-absorbent cotton wool in a drop of alcohol; let most of the latter evaporate, then add sufficient water and cover with a glass. With low power, slender, clear fibres, not very different in appearance from linen fibres, will be seen. Some of them are marked by what appears to be constriction. With high power, long, flat bands, which have caved in, often twisted like a corkscrew, at times striated diagonally, will be seen (Fig. 26, C and D). The fibres do not possess cross-lines. They are the long hairs on the seeds of the cotton plant, Gossypium, the hairs being plant cells, consisting at maturity only of cellulose walls which fall together, giving the fibres the appearance of a flat band. The filaments are about 2 cm. (0.8 inch) long in short staple to 4 cm. (1.6 inch) long in long staple cotton, and about 0.02 mm. (0.0008 inch) broad. There is a central canal running through each fibre, much larger than in linen fibres. The fibres respond to tests for cellulose as in case of linen. With ammonio-copper hydroxide solution, when the swelling action is moderated, there often appear constrictions alternating with large swellings. This is due to the cuticle which covers the surface of the fibres. Linen has no cuticle and does not give the appearance mentioned. The cuticle is insoluble in the reagent, not being cellulose in nature.

Wool.—Mount some fibres from white woolen yarn in water. With low power the fibres are clear, slightly roughened on the surfaces with faint cross-lines. Under high power the fibres are cylindrical, containing a central axial substance, called the medulla (not present in all hairs or wool). The surface is covered by imbricated scales, like tiles on a roof, giving to the edges of the fibres a barbed appearance (Fig. 26, E). Compare a human hair with wool. If raw wool be examined, globules of fatty matter (wool fat) will be seen adhering to the fibres.

Wool being of animal origin does not give the cellulose reactions, as do linen and cotton fibres, but shows protein reactions. Treated with iodine solution and sulphuric acid, as described under linen, the fibres are stained a deep yellow and do not dissolve even when warmed. When warmed on the slide with a saturated aqueous solution of picric acid the fibres are stained yellow; cellulose does not stain with this reagent. Warmed with ammonio-copper hydroxide solution, wool turns bluish-violet and its structure becomes more distinct, but it does not dissolve or swell.

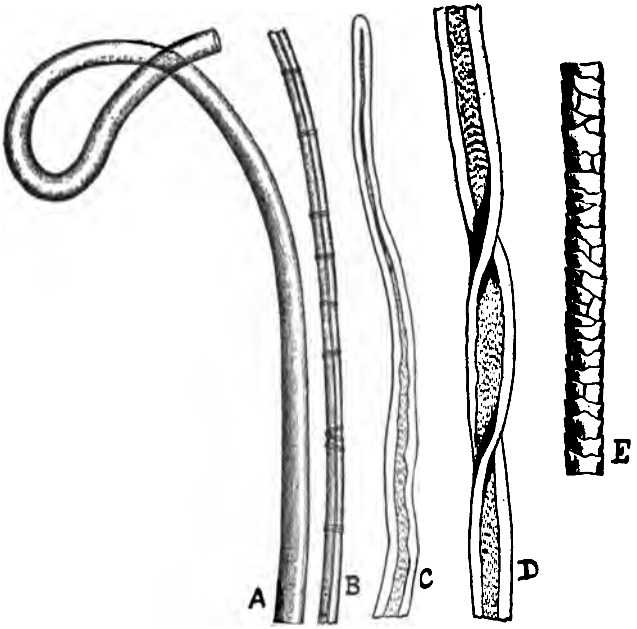


Fig. 26.—A, Silk; B, Linen; C and D, Cotton; E, Wool.

Wool varies in its details of structure, and the identification of its source is not always an easy task, being sometimes well-nigh impossible.

Silk.—Scrape some threads, as in case of linen, and mount in water. With both low and high powers the fibres appear about the same—shining, dense, cylindrical, structureless, without central canal, easily distinguished from all other spun fibres (Fig. 26, A). Silk is animal in origin and gives the same reactions as wool. Several other bast fibres are very valuable in textile industries and present appearances similar to that of linen.

Hemp is derived from Cannabis sativa, jute from Corchorus olitorius and Corchorus capsularis, Manila hemp from Musa textilis. More detailed study of various fibres may be found in some large work, for example, *Die Mikroskopie der Technisch-verwendeten Faserstoffe,* by von Höhnel.

CHAPTER III.

YEAST (TORULA OR SACCHAROMYCES CEREVISIÆ.

This is a plant and is that which causes alcoholic fermentation in sugar solutions. It is a plant of the simplest kind, consisting of a single cell. Plants are divided into four series according to their complexity of structure and functions.

Thallophyta—(Thallus plants)
Bryophyta—(Moss plants) } Cryptogamia or Flowerless plants.
Pteridophyta—(Fern plants)
Spermaphyta—(Seed plants) or Phanerogamia—(Flowering pants).

The Thallophyta are a large group of plants in which there is no clear differentiation of the plant body into root, stem and leaf. A vast number of forms are included, which differ greatly among themselves in complexity, but even the highest forms never have true roots, and in the great majority of cases there is no differentiation into stem and leaves. There is never a clear differentiation into epidermal, fundamental and fibro-vascular systems of tissues as in the ferns and flowering plants.

The Thallophyta are divided into a number of classes, one of which is called *Fungi,* or Moulds. It is thought by some that yeast belongs to the fungi, being a degenerate form. Yeast occurs both *wild* as well as *cultivated,* the former living upon fruits or in fruit juices and occurring in the air, the latter being employed in brewing and for making bread, etc. There are a number of species of wild and cultivated yeasts, and it is probable that cultivated yeasts are descended from similar forms of wild yeasts. Saccharomyces cerevisiæ, or brewers' yeast, is one species, but ordinary commercial yeast seldom consists of this species alone. In recent years the various kinds of yeasts have been grown separately, and they are used for the production of distinct kinds of beer and wine.

Sow some fresh baker's yeast in Pasteur's fluid and keep in a warm place. As soon as the solution begins to froth and the yeast is manifestly increasing in quantity it is ready for study. Fermentation is most active between 28° and 34° C. At 38° C. growth ceases.

Mount a drop of the liquid and examine with low power, minute specks will be seen. With high power numerous rounded or ellipsoidal bodies will be seen either single or loosely united into short chains. The diameter of the cells varies from 1.5 to 15 (average 8 to 10) microns. Each Torula consists of a well-defined homogeneous transparent sac or cell-wall of cellulose material, enclosing a semi-fluid granular substance called *protoplasm,* within which there is often a space full of a more watery fluid than the rest, termed a *vacuole.* The whole structure is known as a *cell.* The cell-wall is comparatively tough, but may easily be burst and the contents thrown out; it is thicker in old cells than in young actively growing ones. Minute shining dots, thought to be fat globules, may be seen in the protoplasm, but there is neither chlorophyll nor starch present. By the use of special reagents, a nucleus has been demonstrated to be present in the cells, but it is never seen in the living cells.

Torulæ break down sugar mainly into alcohol and carbon dioxide gas, and at the same time increase in number. Multiplication takes place in this way. A small protuberance begins to form on the parent Torula, which grows larger, forming a bud. The bud increases until it attains the size of the parent Torula and eventually becomes detached, though generally not until it has developed other buds on itself and these still gemmation or budding are apt to adhere others. The Torulæ produced thus by to each other for a long time and thus produce heaps and strings (Fig. 27).

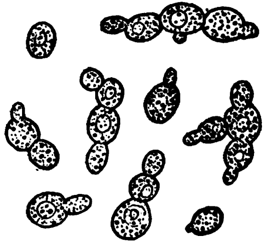


Fig. 27.—Yeast.

Mount a drop of the yeast culture in a small drop of fuchsin stain. Note which cells stain most rapidly and deeply. Actively-growing protoplasm is not stained readily by many dyes, while dead or passive protoplasm is colored quickly by the same dyes. The cell-wall is unaffected and the vacuole also, although the latter may appear purplish, because it is seen through a colored layer of protoplasm.

Make another mount and apply iodine solution at the edge of the cover glass. As the iodine diffuses under the glass the protoplasm of the cells is stained yellowish-brown. This is one of the best tests for protein matter. The absence of blue-stained particles is proof that the cells contain no starch. The cell-wall is not stained.

Yeast occurs in the market in a dry or pasty condition as "yeast cakes." Make an emulsion of a bit of one of these cakes in water and examine a drop under high power. Cells similar or identical to those seen in the yeast culture are in abundance. In the pasty cake there are also present rounded bodies many times larger than the yeast cells. These are starch grains, usually of potato starch, which are added to absorb the water of the mass of yeast cells in order to render it semi-solid and capable of being molded into cakes.

To the drop of yeast just examined, apply a drop of iodine solution at the edge of the cover glass. As it diffuses under the glass the yeast cells stain yellowish brown, the starch grains dark blue.

The usefulness of yeast in bread-making is due to the carbon dioxide gas evolved in the dough. In the latter some starch is converted into grape sugar by the enzyme diastase present in the flour from the wheat grains, and the sugar is decomposed into carbon dioxide and alcohol by the yeast. The carbon dioxide, distributed throughout the mass, cannot escape easily because of the sticky nature of the dough, and thus causes the latter to puff up and become porous when baked. The alcohol is for the most part lost in the baking of the dough.

In brewing industries two well-defined varieties of yeast are used, known respectively as *top* and *bottom* yeast. Top yeast is employed in making English ale, stout and porter, fermentation taking place at ordinary summer temperature and producing carbon dioxide rapidly enough to cause the yeast to collect at the surface of the liquid, hence the name top yeast. Bottom yeast is used in making "lager" beer, and grows quietly at the bottom of the vat at a temperature of about 4° C., which is kept constant by artificial means. Besides this difference in conditions of growth, the two yeasts also differ in form, size and structure when seen under the microscope.

Torula is classed among the plants, because it has a cellulose cell-wall and the power of constructing protoplasm (living matter) out of comparatively simple substances, such as ammonium tartrate, which is distinctively a vegetable peculiarity. But though a plant, it contains neither starch nor chlorophyll, and cannot obtain the whole of its food from inorganic compounds, thus differing widely from green plants.

Pasteur's Solution.—Potassium phosphate, 2 parts; calcium phosphate, 0.2 parts; magnesium sulphate, 0.2 parts; ammonium tartrate, 10 parts; cane sugar, 150 parts; water, 838 parts. To preserve this solution, it must be sterilized, which may easily be done by placing the bottle containing it in cold water, and gradually raising the latter to boiling and maintaining it at that point for about one-half hour. The bottle is first plugged with cotton.

A convenient substitute for Pasteur's Solution is urine, which contains all the mineral matter necessary for plant growth. By dissolving a lump of grape sugar about one-half inch thick in 50 cc. of urine, adding a very small piece of yeast cake and keeping the mixture warm, a vigorous fermentation will take place.

CHAPTER IV.

BACTERIA (SCHIZOMYCETES, OR FISSION MOULDS).

One of the classes of the Thallophyte series of plants is the Schizophytes. This class is composed chiefly of the Schizomycetes or Bacteria. These are extremely low forms of plant life, being exceedingly simple in structure and always minute, some of them being the smallest of known organisms. They are mostly unicellular, or, if consisting of cell-aggregates, as is sometimes the case, the cells are united in a simple way and have very little dependence upon each other. They are the most abundant of organisms. All are chlorophylless, i. e., without coloring matter. The cells agree in having mostly rigid transparent walls and colorless cell-contents, but different species differ considerably in form, size, etc. Their usual mode of increase is by *fission* or splitting, but they also produce very minute so-called *spores,* which, however, are not spores in the same sense as are those of higher plants.

In some species the cells, after fission, immediately become independent; in others they remain united for a time, to form filaments or chains of various lengths. Many of the species in some stage of their development have the habit of secreting a jelly and increasing rapidly by fission, forming large gelatinous colonies. These are called *zoöglœa-masses.* "Mother-of-vinegar" and the so-called "blood-rain," consisting of red gelatinous spots often found on putrefying bread, are examples of zoöglœa-masses.

In all putrefying fluids or solutions that contain decaying organic matter bacteria swarm in myriads. They are, in fact, the inciting cause of putrefaction. By their agency also milk sours, wine is converted into vinegar, etc. So far as animal life is concerned, most of the species are harmless or perhaps even beneficial, while others are the source of some of the most dreaded and most fatal of diseases. Chicken cholera, splenic fever, typhoid fever, diphtheria and leprosy are examples. A peculiar interest therefore attaches to the study of these organisms.

Bacteria are killed at about 70° C. or above, but the spores can, in many cases, survive a temperature above 100° C. Spores are little specialized cells which have the power of developing into cells or plants in all respects like the ones from which they were derived. In the case of bacteria they are formed for protective and not for reproductive purposes; they can withstand conditions of temperature, etc., under which the ordinary cells die and thus ensure the survival of the bacteria.

The spores (or *endospores,* as they have also been named) are formed when unfavorable conditions begin to predominate, and they may be looked upon as resting or passive forms of bacteria, which become active when placed under favorable conditions. Taking the hay bacillus (see below) as an example, the formation of spores begins by the contracting of the protoplasm into an oval mass, usually toward one end of the cell. The spore when completed is shorter than the parent cell and from one-third to one-half as wide. A firm resisting coat or spore membrane encloses the protoplasm. As a rule only one spore is formed in a cell.

There are some species of bacteria which do not produce spores as described above, but produce *arthrospores* in the following manner: A long rod divides into several short rounded bodies which are clear and seem to have the power to resist adverse conditions. There is as yet no good evidence that they can resist the effects of heat as do endogenous spores. Bacteriologists regard them simply as resting cells. They are called arthrospores because they are formed as joints or segments.

A third method of spore formation in a few peculiar bacteria is by the process known as internal cell formation, in which the protoplasm in a large thread divides into many small spherical bodies which finally escape.

Bacteria are conveniently grouped according to their shapes, as illustrated in Fig. 28, in which *a* is the *Micrococcus* or spherical form; *b* the *Bacterium* or rod-like form; *c* the *Bacillus* or filiform form, and *d* the *Spirillum* or coiled form. In growing, bacteria are often grouped in different arrangements, to which special names are applied, thus *Streptococcus,* a moniliform or necklace-like grouping of cocci; *Diplococcus,* cocci in pairs; *Staphylococcus,* single cocci; *Leptothrix,* a filament of bacilli; *Sarcina,* a plate of cocci.

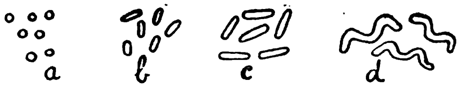


Fig. 28.—Forms of Bacteria.

In size the spheres vary from 0.25 to 1.5 μ the rods may be only 0.3 m. wide or as much as 1.5 to 2.5 μ and in length vary from a fraction of a micron to long threads.

Make an infusion of fresh hay by steeping it in water warmed to between 40° and 50° C. for one-half hour or more, filter and set aside for 36 hours or more The liquid becomes cloudy, due to swarms of bacteria. Examine a drop of the liquid under the highest power at command. *Focusing must be done very carefully because of the extreme minuteness and transparency of the* *cells*. The illumination must be somewhat dimmed, else the bacteria will be almost invisible in the glare. A convenient way to find the focal plane is to move the slide about until a coarser object, like a speck of dust, comes into view, and with this as a guide to make the fine adjustment of the focus.

Note the moving bacteria, elliptical or rod-like, sometimes forming short, jointed rows. The cells have an outer, more transparent wall, enveloping a more opaque substance.

Apply a drop of iodine solution at the edge of the cover glass. The bacteria are killed, all forward motion ceases, the contents of the cells are stained and become more conspicuous. The cell-wall does not stain. Other forms that may be found are micrococcus, bacillus and spirillum.

Place a small drop of the infusion in the center of a cover glass and spread it into a uniform thin layer with a needle. Set it aside until *dry*, and then pass the glass (film side up) three times through the flame of an alcohol lamp or Bunsen burner by means of a pair of forceps, each passage through the flame taking about one second. This fixes the bacteria to the glass. Cover the film for 10 to 15 seconds with carbol-fuchsin, or gentian violet, or methylene blue (see Appendix for preparation of stains). Wash in water, dry in the air high over a flame, and mount in a drop of Canada balsam on a slide. Draw the different forms of bacteria brought out by the stain.

To secure various kinds of bacteria, infusions and decoctions should be made of various kinds of leaves, cabbage leaves, beans, potatoes, etc. Set these aside for a few days and examine them.

Resting Bacteria or Zoöglœa Stage.—The hay-infusion after a time develops a scum or zoöglœa. Mount a little of this and examine with high power. Myriads of bacteria resting in a gelatinous mass will be seen. Although they do not move away from their places, the bacteria will be seen to have a wiggling or oscillatory motion, known as the *Brownian movement*. This motion is not a vital one, but is characteristic of very small bodies, whether dead or alive. Fine clay, pumice, lamp-black, gamboge, show the same motion. The cause is not definitely known.

Raise the cover glass and add a drop of iodine solution to the scum. The bacteria stain, but not the gelatinous material in which they are imbedded.

Mount a little of the scum composing "mother-of-vinegar." Nearly the same appearance will be seen as in the case of the scum of the hay-infusion. The scum is known as *mycoderma aceti*, and the bacteria cause the oxidation of dilute alcohol to acetic acid (vinegar).

In order to show the position of the plants already studied, as well as those to follow, in the system of classification of plants, a table of the Thallophyta, with the subdivisions, is here appended.

Thallophyte Series.

Class	*Sub-Class*	*Group*	*Genus*
1. Myxomycetes.			
2. Schizophytes.	1. Cyanophyceæ.		Glœocapsa. Nostoc.
	2. Schizomycetes. (Bacteria.)		
3. Algæ.	1. Chlorophyceæ.	1.	
		2. Protococcales.	Pleurococcus. Hydrodictyon.
		3.	
		4.	
		5. Conjugales.	Zygnema. Spirogyra.
		6.	
	2. Phæophyceæ.		
	3. Rhodophyceæ.		
4. Fungi.	1. Phycomycetes.	1.	
		2. Zygomycetes.	(Mucor.)
	2. Ascomycetes.	1. Protoascales.	Saccharomycetes.
		2	
		3	
		4	
		5	
		6. Plectascales.	Penicillium.
		7. Pyrenomycetales.	(Mildew.) (Ergot.)
		8.	
	3. Basidiomycetes.	1.	
		etc.	
		9. Agaricales.	(Mushroom.)
		etc.	
		14.	
5. Lichenes			

The table is only partly completed and only those sub-divisions are given with which the plants studied in these lessons are concerned.

CHAPTER V.

PLEUROCOCCUS VULGARIS (PROTOCOCCUS VIRIDIS).

Pleurococcus grows upon the surface of various objects and is often so abundant as to give them a pronounced green color, especially when found on the north side of old tree trunks or fences.

Examine a piece of bark or wood bearing the plant, observe the color both before and after moistening with water, also the evenness with which it covers the surface of the object. Gently scrape the plant off into alcohol and observe the green color gradually imparted to the alcohol by the *chlorophyll*—the coloring matter of the plant.

Carefully scrape off a little of the green powdery layer and mount in water, avoiding air bubbles. Students usually scrape too roughly and too much material, both of which faults must be avoided. The plant can also be removed from the object by moistening and loosening the green granular material with a camel's-hair brush, and transferring it to a drop of water on a slide. With low power, a mass of greenish dust-like particles of varying sizes will be seen.

With high power, divers pictures are presented. There will be seen a number of single cells perfectly round in shape; also groups of two, three, or four cells, respectively, in which the cells have varying degrees of contact with one another; finally colonies of a number of loosely attached individual cells (Fig. 29).

Make drawings of the various forms found, illustrating the method of cell multiplication.

Pleurococcus multiplies by the process known as fission, which takes place thus. The cell elongates and the protoplasm divides into two masses across its longer axis. Between these masses a partition forms, subdividing the cell, and the halves either separate at once and become round and independent cells, or one or both halves again divide in a similar way (the wall of division, however, being at right angles to the first wall) before they separate, and thus groups of three or four cells are formed.

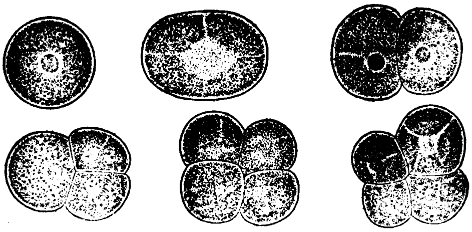


Fig. 29.—Pleurococcus (after Cohn).

Note the colorless cell-wall surrounding each cell and the more or less granular cell contents colored green by chlorophyll.

Stain with iodine and note that the protoplasmic contents stain brownish-yellow. By carefully staining with dilute chlor-iodide of zinc, the chlorophyll bodies, consisting of two to several closely packed granules and forming the green part of the cell, and the nucleus, may be seen.

Pleurococcus is a unicellular plant, but, unlike the yeast plant, it contains chlorophyll bodies, which latter are able to convert inorganic food materials (carbon dioxide and water) into organic compounds. This function is lacking in the yeast plant.

GLŒOCAPSA.

If this plant can be obtained, it will prove intéresting to compare it with Pleurococcus. It belongs to the Cyanophyceæ, a sub-division of the Schizophytes, whereas the Pleurococcus belongs to a sub-division of the Algæ.

A favorite place for the growth of Glœocapsa is on old flower pots in green-houses and on damp rocks near springs. It forms slimy masses.

The method of reproduction is somewhat like that of Pleurococcus. The cells may be observed in various stages of fission, but they are held together in a gelatinous matrix which is derived from the cell-walls. This jelly-like formation from the walls and the bluish-green color of the plant body are characteristic of the group to which Glœocapsa belongs.

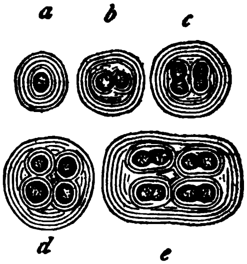


Fig. 30--Glœocapsa, showing thickened, stratified, gelatinous cell-walls, and mode of multiplication (Bastin).

NOSTOC.

Nostoc should also be examined. It, like Glœocapsa, belongs to the Cyanophyceæ. Nostocs occur as greenish or brownish gelatinous masses in ponds or slow streams, or in damp places next to rivers, swamps and lakes or in green-houses. The individual cells are very similar to those of Glœocapsa, but in Nostoc as the cells divide, filaments are formed in which the cells are so slightly touching, that the filaments resemble strings of beads. At intervals along the filaments occur peculiar cells called *heterocysts,* which are devoid of all color and somewhat larger than the other cells. By these the filaments are marked off into distinct sections called *hormogonia,* each of which is capable of establishing a new colony.

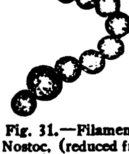


Fig. 31.—Filament o Nostoc, (reduced from Bastin).

Nostoc may also develop from so-called arthrospores. Upon the approach of cold weather or any other unfavorable period, certain cells of the filament enlarge, accumulate reserve food, and become thick walled. These cells are able to endure the cold, etc., and upon the return of favorable conditions the heavy wall is ruptured and a new filament arises from it.

In the study of these low plants we observe how a beginning is made in the formation of more complex tissues. A cell that divides only in one plane can give rise to strings of cells, or to filaments; division in two planes results in surfaces; but division in three planes results in masses of cells.

CHAPTER VI.

SPIROGYRA.

This plant belongs to the third class of the Thallophyte series, which is known as Algæ. The class includes nearly all the Thallophyte plants which contain chlorophyll, in virtue of which they are able to assimilate the carbon dioxide of the air, or of the water in which they grow, in the same way as do the more complex green plants.

The Algæ embrace plants of the greatest diversity of form and structure, from the minute Pleurococcus found on tree trunks, to the giant kelps of the ocean, which frequently attain lengths of 500 to 1000 feet. Those that live in fresh water for the most part are an assemblage of quite simple plants, none of the members attaining any great degree of complexity. The plant body consists either of single cells, or of an elongated filament composed of united cells; sometimes, however, they form surfaces, and in other cases the plants are aggregated into communities. In these plants we find the first examples of undoubted sexuality, and throughout the group the organs and methods of fertilization are nearly enough uniform to enable us to use them as distinguishing characters. They abound in ponds and slow-running streams.

Spirogyra will illustrate some characteristics of the class (Fig. 32). Its position in the system of plants is given in the table (Chapter IV). It belongs to the group Conjugales. This group differs from all other Algæ in the peculiarly complex structure of the chlorophyll bodies and the mode of sexual reproduction (except some of the Diatomaceæ) which consists in the direct conjugation or union of two ordinary vegetative cells, hence the name *Conjugales.* Spirogyra is a filamentous plant, very common in ponds and ditches as a green scum composed of silky, green threads, which sometimes attain a length of six or eight inches. The filaments are unbranched and composed of a row of cylindrical cells all alike and independent of each other and loosely joined together. The name is given in allusion to the fact that the chlorophyll bodies, i. e., the bodies bearing the green coloring matter, form spiral bands winding around the cell on the interior of the cell-wall. Sometimes the bands are single, at other times double or treble (Zygnemas have stellate chlorophyll bodies, two in each cell, arranged axially). At intervals along each band are to be seen highly refractive lenticular bodies called *pyrenoids*. When exposed to the light for some time the pyrenoids would be found, on appropriate treatment, to be surrounded by starch grains.

The cells are bounded by well-marked, refractive cellulose walls. Next to the wall is a thin layer of protoplasm, better seen by staining with iodine solution. The chlorophyll bands are in contact with this layer of protoplasm. The greater part of the interior of the cell is occupied by a large vacuole containing *cell sap*, i. e., water with substances in solution. Each cell has a usually centrally-placed, distinct protoplasmic body known as a *nucleus*, with radiating extensions of protoplasm passing from it to the outer layer of protoplasm next the cell-wall. The growth of Spirogyra in length is brought about by cell division. Each cell is repeatedly divided into two equal parts by the appearance in it of a cross-partition. This process takes place during the night, and special precaution must be taken in order to study it. This method of cell formation is the general mode throughout the vegetable kingdom.

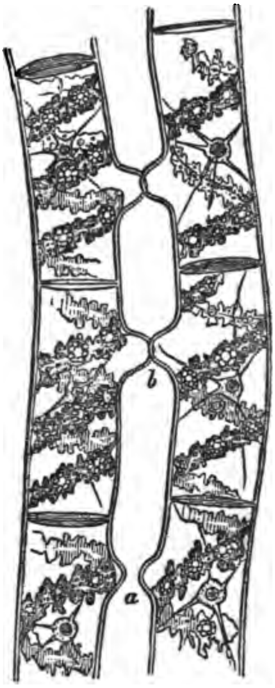


Fig. 32.—Spirogyra longata (Bessey).

The method of reproduction in Spirogyra is a sexual one and known as conjugation. This process occurs from early spring to June and July, but can be induced when the plant is under cultivation by allowing the water in which it is growing to slowly evaporate. Two filaments arrange themselves side by side, and the cells lying opposite each other send out each a process or tube; these unite and the protoplasm from one cell passes over and coalesces with that in the cell opposite. In Fig. 32 two such tubes about to unite are shown at b, while the beginning of formation of two other tubes is shown at a.

The conjugating cells are called *gametes* and the result of the process is a new cell called a *zygospore*. This is set free by decay of the walls of the old cell and falls to the bottom of the water and rests until proper time for growth.

It is not an easy matter to find conjugating forms of Spirogyra, and their study is not well suited for class work.

There are a number of species of Spirogyra, and the student should keep a lookout for different kinds of filaments in the specimen studied. Mount some filaments in water and, under low power, note the great length of the filaments, as well as of the individual cells, the uniform diameter, the well-defined cell-walls and the conspicuous green chlorophyll bands, the shape and relative length and breadth of the cells.

With high power, note more especially the crenulated and wrinkled margin of the bands and the refractive nodules at intervals along them (*pyrenoids*). Look for a nucleus. This is sometimes hidden by the chlorophyll bands and then not easily seen, but there will always be found some cells in which the nucleus stands out clearly.

Apply iodine solution. The bands stain deeply, especially the dense pyrenoids, which appear almost black. The nucleus also appears much more conspicuous when stained.

It may not be possible to obtain Spirogyra in a growing state at the time it is wanted for study. In such a case it should be collected at some other time, when a supply is found, and preserved for future use in dilute formaldehyde solution (one volume of 40 per cent. "formalin" to 10 or 15 volumes of water). In this solution the color is bleached only slowly, and if the specimen has not been kept too long it will still show a green color.

Another large-sized Alga often found in slow streams is the *Hydrodictyon* or *Water-net*. The cells are united into a network, which has the shape of an elongated bag, often 8 or 10 inches long. The cells are quite large, cylindrical and filled with dense granular chlorophyll matter. The ends of three or four cells meet at a common point. Several nets may be entangled in one another, representing different generations, the cells of the younger nets being much smaller than those of the old and matured nets.

The structure is best studied under low objective in a watch glass containing water, as thus crushing of the net is avoided, which would happen under a cover glass. For convenient handling, Spirogyra and Water-net may be cut up with a pair of scissors into suitable pieces.

CHAPTER VII.

REPRODUCTION.

This is the power that plants possess of giving rise to new individuals, and the process takes place by one of three ways, namely, Division, Rejuvenescence and Union. The first two modes are *asexual,* the last *sexual.*

There are three varieties of reproduction by division:

DIVISION. { Fission.
Gemmation.
Internal cell formation.

FISSION.—The most common mode of division. This is the separation of a cell into equal portions.

a. A constriction takes place in the middle of the cell and along the plane of this constriction, the cell-walls may grow inward until the cell contents become separated into two equal portions, the process reminding one of the gradual closing of an iris diaphragm. This mode has been observed in some of the lower Algæ (Spirogyra).

b. A delicate partition of cellulose may be formed through the middle of the cell. This is the usual mode by which "tissues" are formed and growth takes place in all the higher plants. (See Cell Division, page 59).

GEMMATION.—This method is found in the yeast plant and its relations. (See Torula for description.)

INTERNAL CELL FORMATION.—The protoplasm of a cell breaks up into two or more rounded masses, each of which eventually acquires a cell-wall of its own and escapes from the parent cell by the rupture or decay of the old cell-wall. Example, spores in Mucor, ascospores in Lichens and some Fungi, pollen grains in the anthers of flowering plants.

REJUVENESCENCE.—The protoplasm aggregates into a rounded mass, escapes through the cell-wall and subsequently forms a new cell-wall. Commonly before the new cell-wall forms, the protoplasm forms cilia and moves about. Rejuvenescence is found only among lower forms of plant life; for example, Oedogonium, one of the Algæ.

As was said above, Division and Rejuvenescence constitute asexual reproduction. Asexual reproduction takes place either vegetatively, or by means of asexual spores. In the former method the parent plant throws off from itself ordinary vegetative cells; in the latter, specialized cells called spores are formed. Examples of the first are bacteria, yeast, Pleurococcus, cell multiplication in higher plants, multiplication in case of many plants by bulbs, tubers, stolons, offsets, etc.

Spore reproduction by the asexual process is exemplified in many flowerless plants. Examples, basidio-spores on the gills of the common mushroom, spores of Mucor, spores on the under side of fern leaves, conidia of Penicillium, etc. Spores are commonly borne in a special organ called a *sporangium.*

UNION OF CELLS.—SEXUAL REPRODUCTION.

This consists in the coming together and blending of the protoplasm of two distinct cells to form a new one.

a. The uniting cells may be alike and the process is then known as *conjugation;* this mode is found only in certain low forms of plant life, as Mucor (a mould), Diatoms, Spirogyra, Desmids, all of which, except the first, belong to Algæ.

b. The uniting cells may be unlike, the process being then known as *fertilization.* One cell (the male or sperm cell) is commonly not only smaller, but more active than the other (called the female or germ cell). Examples, many of the Algæ, the sexual generation of all the mosses, ferns, and higher plants.

The uniting cells, as in the case of conjugation, are called gametes. A gamete cannot by itself reproduce the plant. The result of the union of the sperm and egg cells is a *sexual spore,* which may either be a resting spore, as in Spirogyra, or it may germinate (develop) at once into an embryo, as in seed plants.

RÉSUMÉ OF REPRODUCTION.

- REPRODUCTION.
  - ASEXUAL
    - DIVISION
      - FISSION.
      - GEMMATION.
      - INTERNAL CELL FORMATION.
    - REJUVENESCENCE.
  - SEXUAL—UNION
    - CONJUGATION.
    - FERTILIZATION.

CHAPTER VIII.

MOULDS (FUNGI).

Moulds belong to the class of plants known as Fungi, which latter, as we have already seen, form one of the divisions of the Thallophyta. The Fungi are, in their habits, chlorophylless *saprophytes* or *parasites.* (A saprophyte is a plant which derives its sustenance from decaying organic matter. A parasite lives on other organisms.) In all but a few instances (see Torula), their vegetative parts consist of slender segmented or unsegmented, usually colorless filaments, each one being known as a *hypha.* These ramify among decaying organic debris or invade the tissues of living organisms, plant or animal, and derive their sustenance from them. In the simpler hyphal forms the hypæ occur singly or more or less interwoven into a tangled felt-work, but they are not gathered into definite forms and have little or no dependence on each other. In the higher groups, however, there is more or less division of labor among the hyphæ, and they become consolidated into false tissues, which acquire definite shapes according to the species. Of this character are the so-called fruits which constitute the above-ground parts of the agarics, puff-balls cup-fungi, etc. and the *sclerotium,* a compact hard mass of thick-walled hyphæ, which serves as a resting stage in the development of some species, for example, *Ergot of rye.*

Fungi reproduce asexually by means of spores variously named, according to their mode of origin, conidia, ascospores, basidiospores, zygospores, æcidiospores, etc. These are as a rule, thick-walled cells, which become separated from the parent hyphæ in ways which are more or less characteristic in the different groups. In all hyphal Fungi the hyphæ consist of two portions—the *vegetative,* which ramifies in the substratum, often forming tangled, felt-like masses of threads called the *mycelium;* and the *reproductive,* which comes to the surface. The latter produces the spores; condia, in the bread-mould, Penicillium, basidiospores, in the common mushroom, Agaricus campestris, etc. The plate-like bodies or gills on the under surface of the cap of the mushroom constituting the hymenium, or spore-bearing surface.

In a large number of Fungi, including some of the most highly organized forms, sexual reproduction is unknown. In other species, sexual reproduction takes place. The sexual reproduction of Fungi is exceedingly varied.

PENICILLIUM GLAUCUM (COMMON GREEN MOULD).

This mould is familiar to everyone from its forming sage-green crusts upon bread, jam, old boots, etc. It may be obtained at any time by placing a moist piece of bread under a bell-jar in a moderately warm place. When spores appear, sow some in Pasteur's fluid (see under Torula). Moulds growing in this fluid are easier to examine than when growing on bread. On examining a patch of mould on the surface of the fluid, it is found to consist of a horizontal felt work of delicate tubular filaments, the hyphæ, forming a crust like so much blotting paper, which is known as the *mycelium*. Hyphæ project from this into the air, b, and bear a green powder, the spores, c, Fig. 33. These hyphæ are called *aerial*. From the mycelium other hyphæ grow down into the liquid and are called *submerged hyphæ*, corresponding somewhat to the roots of higher plants.

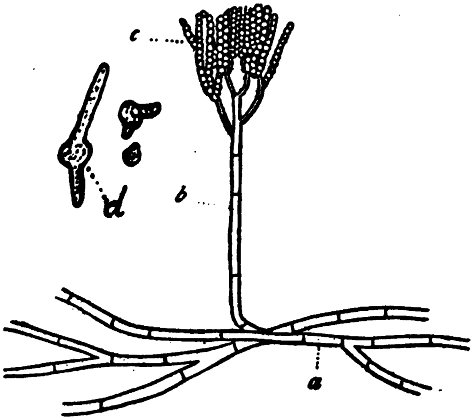


Fig. 33.—Penicillium glaucum.

Carefully make a thin section of the mycelium by cutting between two pieces of pith. Moisten a section on a slide with alcohol, allow the latter to nearly evaporate, then add water and cover. Examine first under low power and observe the appearances described above.

With high power, observe that each hypha has a transparent wall and protoplasmic contents and is divided by transverse partitions into a number of cells. Each cell has several large clear spaces, the vacuoles and a number of nuclei which, however, are only visible by staining properly.

The hyphæ frequently branch and are inextricably entangled with one another, but every hypha with its branches is quite distinct from every other one. If the section be a little too thick and obscure, gentle tapping on the cover glass over the section will spread the parts, so that they may be more easily seen.

Note the aerial hyphæ, with brushes or branches, which become constricted on their ends into a series of rounded spores like a row of beads. These hyphæ which bear the spores or conidia are called *conidiaphores*. The conidia form the loose green powder characteristic of the mould. The spore is a round, transparent sac enclosing a mass of protoplasm and is in all essential respects similar to a yeast cell. When sown in an appropriate medium (Pasteur's solution) it germinates and forms hyphæ from several points, forming a new plant like the original one (Fig. 33, d).

Stain different sections with fuchsin, hæmatoxylin and iodine and note results.

Besides Penicillium, usually other moulds will be found on mouldy bread or other matters. The most prominent among them will probably be *Eurotium Aspergillus glaucus,* which may be distinguished from Penicillium by its higher growth, less velvet-like appearance and the olive-green color of the spores. The conidiaphores of Eurotium are about $^1/_{16}$ inch long, visible to the eye, and bear roundish white (unripe) or pale-green heads closely packed.

A pure growth of either of the fungi described above may be obtained as follows: Place a few thoroughly boiled (and thus sterilized) French plums on a sterile glass plate and infect them with spores taken from as pure a patch of the mould as can be found by a previously heated and cooled needle. Cover the plate with a sterile bell glass and keep in a moderately warm place. (The plate and bell glass may be sterilized by placing them in an air-bath heated to 105° or 110° C. for one-half hour or more.)

Both Penicillium and Eurotium also form ascospores.

RHIZOPUS NIGRICANS (MUCOR STOLONIFER).

THE BREAD MOULD.

This mould may be grown by keeping some bread very moist and warm under a bell jar or by placing some moist Poke-root in a bottle and closing. Mucor is similar to Penicillium in its vegetative growth; its reproduction is somewhat different. From the mycelium arise erect, aerial hyphæ, each one bearing a rounded, dark head or spore case, looking like a pin head and called a *sporangium.* The wall of the spore case is beset with minute asperities of oxalate of lime, and inside the case are a great number of minute oval bodies, the spores, held together by a transparent intermediate substance. When ripe, the thin and brittle coat of the case bursts at the slightest pressure, setting free the spores. A little portion of the wall of the spore case remains adhering to the stalk as a collar. The cavity of the stalk does not communicate with the sporangium, but is cut off by a bulging partition, forming a central projection known as the *columella.* This may be mistaken for the spore case itself.

The spores are oval and larger than those of Penicillium, consisting of a sac enclosing protoplasm and a nucleus. When sown in a proper medium they send out hyphæ and produce a new plant. The spores are at first colorless, but when ripe are colored and give the black appearance to the sporangia.

The hyphæ are cylindrical threads, longer and larger in diameter than those of Penicillium, and when young have no dividing partitions, so that each hypha, however long, with all its branches, forms a single cell. In old growths, partitions may be found after the production of sporangia. The hyphæ contain granular protoplasm with vacuoles and nuclei.

Carefully remove some hyphæ, white (unripe) and dark spore cases with a teasing needle or forceps to a slide and keep them spread apart. Add a drop or two of 70 or 80 per cent. alcohol and cover. Water causes the spore cases to swell and burst, hence should not be used. Examine with low and high power. Note the hyphæ, spore cases (both whole and broken), columella, spores, protoplasm, etc. Also apply fuchsin and iodine stains and note the effects. Compare with Penicillium as to its dimensions and parts.

Mucor also reproduces itself sexually, by conjugation.

When moist bread is allowed to become mouldy, Mucor is apt to be the first growth. Later on this mould will die away and Penicillium or Eurotium will get the upper hand and flourish. The source of the mould is the spores which float about in the air or are present in water.

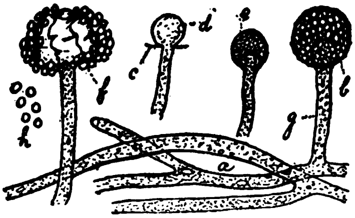


Fig. 34.—Mucor stolonifer. a, hypha; b, ripe spore case; c, collar or remains of broken wall of spore case; d, columella or dome-like partition separating spore case from the cavity of its stalk, g; f, broken spore case with spores; e, young spore case, spores not yet formed; h, spores.

MICROSPHÆRA (LILAC MILDEW).

Mildews are very common parasitic Fungi found growing upon leaves of various seed plants. The mycelium spreads over the surface of the leaf like a cobweb. Here and there small dish-like *haustoria* penetrate into the cells of the leaf and absorb their contents.

A very common mildew, Microsphæra alni, may be found growing upon lilac leaves.

To study all the stages of this fungus, leaves should be gathered during the latter part of June, early part of September, and just before the leaves fall.

Examine the leaves first gathered, notice their powdery appearance caused by the abundant conidia or asexual spores formed by abstriction, as in Penicillium. Scrape some of the fungus from the surface of these leaves, and observe under high power. Make a drawing of some of the mycelium, its branching, the irregular diameter of the filaments and the rarity of partition walls. If some of the haustoria are seen draw them also, and also one or two conidiaphores and conidia.

Now examine the leaves gathered last. With naked eye, observe the minute dark disks or *ascocarps;* each one being a little sphere suggested the name Microsphæra.

Prepare a slide with ascocarps and observe their shape, reticulated surface due to cellular structure and appendages extending out from all sides. Draw an ascocarp with its appendages, and observe their dichotomous branching.

Next, crush one while looking through the microscope by pressing on the cover-glass with a needle. Observe the escape of small sacs or *asci,* containing spores. Note the number from each ascocarp, their general shape, the short stalk by which they were attached, and the number and shape of the spores (*ascospores*) in each ascus. Draw an ascus with its spores.

The ascocarp is the result of fertilization. When the production of conidia ceases, the sex organs appear (*oögonium* and *antheridium*). With the fusion of the male nucleus with the female nucleus, the oögonium becomes a short filament or *ascogonium.* In Microsphæra the terminal cell of the ascogonium gives rise to hyphæ which produce several asci. From the cell beneath the oögonium, sterile hyphæ arise which form the sheath of the closed ascocarp, and from this sheath arise the appendages with their dichotomous tips.

Careful examination of the second gathering of leaves may reveal the sex organs.

If willow blight, Uncinula salicis is on hand, compare it with lilac mildew.

CLAVICEPS PURPUREA (ERGOT OF RYE).

Claviceps is a genus of the Fungi whose different species produce Ergot grains on various kinds of grasses. The hyphæ of the species Claviceps purpurea begin their development on the surface and interior of the ovary of the flowers of Rye as delicate filaments. After a certain time the fungus begins to form a dense mass of thick, hard, dark purple hyphæ, which gradually destroy and take the place of the cells of the ovary until finally there is scarcely anything left of the latter. This hard mass, known as the *sclerotium* stage, constitutes the official Ergot of Rye. This is a resting stage, the grain lying dormant until spring, when, if placed in warm, damp soil, there arises a number of stalked bodies with globular heads in which spores are produced. If these spores be carried by the wind to the flowers of Rye they develop and produce new grains in the manner just described.

Wrap a few large grains in moistened filter paper and keep in a corked bottle for several hours. By this time they will have changed from a brittle to a flexible state. Place half of a grain between the two halves of a piece of elder pith, clamp in a microtome and cut very thin transverse sections through the pith and place them in water. Mount in water on a slide and examine with low and high power. The margin of the section consists of smaller cells with brown contents. Within this border the cells are larger and lighter in color, rounded or oval, having thick cell-walls and oily contents. In chloral hydrate solution the cells become clearer and the oil collects in larger globules. In

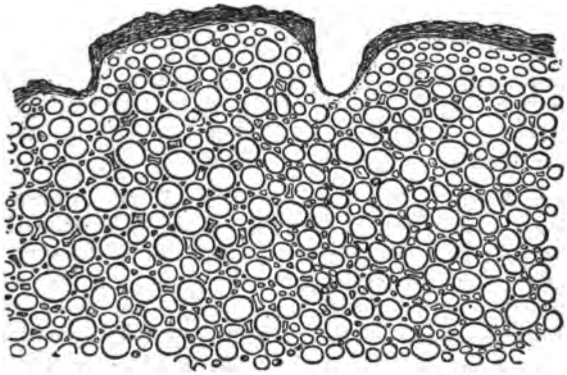


Fig. 35.—Ergot of Rye, Transverse Section (Vogl.)

longitudinal section the appearance is nearly the same as in the transverse. The appearance of these sections is very similar to true parenchyma tissue (see Chapter XI.). False tissue of this kind is known as *pseudo-parenchyma.*

CHAPTER IX.

THE TISSUES OF THE HIGHER PLANTS.

The lessons thus far have been given to the study of some of the simple plants, for the purpose of giving an idea of the nature of the lowest forms of plant life as well as familiarizing the student with the use of the microscope and the manipulation of objects on the slide. Some of the plants studied play an important role in the life economy, for example, yeast, bacteria, moulds, and thus deserve close study. While studying these plants we have learned what is meant by a plant cell, and the subsequent lessons will be devoted to a study of the various kinds of cells and webs of cells known as "tissues," found in the most highly developed and complex plants, the Phanerogamia or flowering plants.

The peculiarity of these is that there is a great division of labor, with corresponding tissues and organs, which have been differentiated from a fundamental tissue. Thus we have a leaf, an organ for manufacturing the food of the plant; the flower, which is the reproductive organ; the stem, the channel for conveying sap; the roots for imbibing water with its dissolved salts. The cells are differentiated into distinct tissues. On passing down to the lower series of plants these tissues become simpler until, finally, in the Thallophyta and most of the Bryophyta we have no distinction of tissues at all. Those plants consist of a homogeneous mass of cells, as we have seen in the case of Algæ and Fungi.

The various tissues or cell-webs of the flowering plants, namely, epidermal, ground, fibro-vascular, stony, etc., are all derived from cells that were at one time alike. By various physical and chemical modifications the cells come to differ from one another and thus to give rise to the different tissues. The cells of stony tissue, as found in shells of nuts, were once like the soft cells of a leaf, but they became subsequently hardened and modified.

A *cell* has been defined as a *nucleated mass of protoplasm.* It may or may not possess a cell-wall different in composition. With rare exceptions in vegetable cells such a wall is present, while most animal cells are destitute of it; but in all essential respects animal and vegetable cells resemble each other. Cells are the structural units of the organism. All plant bodies are composed of cells or of these together with the products of cell activity. Within the compass of the cell occur all those essential phenomena which are called *vital;* the life of a plant resides in its cells; the sum of the activities it exhibits is the sum of the activities of its component cells.

Vegetable cells are, on the average, not more than one five-hundredth or one six-hundredth of an inch in diameter, though in some cases they are large enough to be distinctly seen by the unaided eye, as in the flesh of the Watermelon and the pith of Elder; in rare instances, as the internodal cells of Chara, they may even be more than an inch long. Some cells, on the other hand, are so small as to be barely visible under the highest powers of the microscope, for example, some bacteria.

The primary form of cells appears to be that of a sphere or spheroid, but commonly, especially in the tissues of the higher plants, they acquire forms quite different from this, and within the limits of the same organism the shapes may be exceedingly various. This may be due to mutual pressure, to unequal growth caused by the unequal operation of various physical forces, as gravitation, light, etc., or to other influence. Cells, like the organs of which they are components, undergo many modifications of form and structure, adapting them to different uses. The cells which make up the body of a plant are comparable to the human units which make up society. A plant is a community or republic of cells, and, to understand it, one must understand the individuals that compose it.

TYPICAL VEGETABLE CELL.

As all the different kinds of cells that go to make up the various tissues of a plant are derived from cells that are at one time all alike, we will begin by a consideration of these primitive or *typical* cells, and afterwards study the various modifications.

Peel off the skin or *epidermis* from the convex surface of an onion scale by making a cross incision and catching the skin between the thumb and the knife or razor edge. Be careful not to draw along with the epidermis any of the thick underlying flesh of the scale. Mount a piece of the skin about a quarter-inch square in water on a slide, cover carefully with a glass so as not to include any air-bubbles.

Examine with low power. Very little will be made out. There is a fine and somewhat irregular network. This is due to an aggregation in a single layer of a number of cells, the network of lines being the bounding cell-walls, which are so nearly transparent as to be almost invisible. If the light be properly dimmed there may be seen in each cell a small denser-looking body which is called the nucleus, and perhaps faintly granular matter. The cells are filled with a semi-liquid matter, which, however, is too transparent to be seen.

Examine the various parts of a cell with high power. The details are somewhat difficult to make out because of the transparency of the cell contents. This is very often the case with living cells, but the difficulty can be overcome by killing the protoplasm or staining it.

Raise the cover glass, add a drop of iodine stain and allow a few moments for it to penetrate the cells, then cover and examine again.

The cell-walls, scarcely stained, are distinctly visible. In mature cells aggregated to form tissues, the common cell-wall between two cells is made up of two like portions separated by a layer of a slightly different chemical substance, which is more soluble in reagents than the rest of the wall and shows different reactions with test reagents and is known as the *middle lamella.* Next to the cell-wall is a layer of protoplasm, granular and deeply stained yellowish brown, called the primordial utricle. Somewhere within the cell will be seen a dense body, the nucleus, surrounded by protoplasm and connected by strings of protoplasm with the utricle. Between the strings are *vacuoles,* clear spaces filled with cell-sap. The nucleus contains several smaller bodies, which are little nuclei or *nucleoli* (plural of nucleolus). In some cells the protoplasm may have shrunken away from the cell-wall at one end, leaving a clear, apparently empty space. The cells dovetail into one another, leaving *no intercellular spaces,* but forming a close impervious layer.

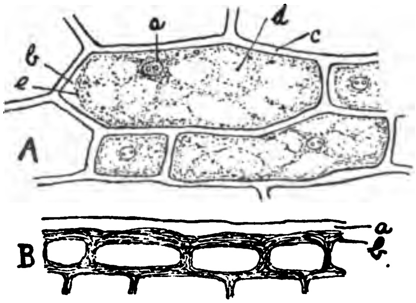


Fig. 36.—Cells of Onion epidermis. A, surface view. a, nucleus; c, cell-wall; d, vacuole; b, space where protoplasm has shrunken from cell-wall; e, primordial utricle. B, cross-section. a, cuticle; b, cellulose portion of cell-wall.

Closer study of the protoplasm will show that it is more or less granular in appearance. These granules are the *plastids,* the transparent medium which surrounds them is the *cytoplasm*. The plastids in the case of the onion cells are colorless; in leaves plastids of a green color are found; in the root of the carrots, they are reddish-yellow. Plastids are therefore classified as *leucoplastids* when colorless, *chloroplastids* when green, and *chromoplastids* when of a color other than green, as yellow, red, or orange.

Like epidermal cells in general, the cells just described are rather flat, but that is not apparent in surface view. This fact is brought out in sections cut vertical to the surface of the epidermis, which is best done by cutting through several scales of the onion. The appearance is given in Fig. 36. The cells are oblong in shape and the outer wall is somewhat thickened.

Raise the cover glass from the section that was stained with iodine, remove the excess of fluid and add a drop of sulphuric acid (2 vols. conc. acid to 1 vol. water), after a moment replace the cover glass and examine. The cell-walls are stained a deep blue, proving that they are cellulose in nature. A light line, *not* blue, but of a yellow color, is in the middle of the cell-walls and marks off the boundary of each cell. This line is the middle lamella. It is composed chiefly of calcium pectate. The cellulose portions, as well as the middle lamella, gradually swell, finally dissolve and disappear.

If a cross-section of the epidermis be treated as above described the thickened outer wall will be seen to consist of two layers, an inner one, which is cellulose and stains blue, and an outer one, which stains yellowish or brownish. This outer layer is called the cuticle and is composed of a substance known as *cutin* (cork substance), which does not dissolve in the acid. Cutin is very resistant to reagents and thus forms an excellent protecting layer. The cuticle is represented in Fig. 36.

The typical cell just described is somewhat advanced from the earliest stage of a cell, known as the *primordial meristem* cell. In such very young cells the wall may not yet have been secreted, or if already formed, it is exceedingly thin and apparently homogeneous, the vacuoles are absent and the entire area enclosed by the wall appears to be filled with protoplasm (Fig. 37). As the cell grows older its wall becomes thicker and differentiated, as described above. By the expansion of the wall the cavity of the cell increases faster than the contained protoplasm, the latter imbibes more water than it is capable of holding in solution and thus sap cavities or vacuoles are formed, which, at the maturity

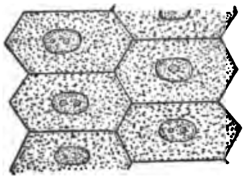


Fig. 37.—Very young cells.

of the cell, often occupy more space than the protoplasm itself. Finally, when the cell is quite old its living contents disappear altogether and the cell is dead matter. During the maturing process, cells become differentiated according to the function to be performed, into epidermal tissue, conducting tissue, fibres, etc., etc.

As the cells develop to form the various tissues, a number of substances make their appearance in the contents. Some of these are chlorophyll, aleurone grains, starch, fatty oils and fats, calcium salts, glucosides, alkaloids, sugar, bitter principles, tannin, resins, gums, inulin, etc. Some of these will be studied in later lessons.

Cell Division.—Every living cell owes its origin to some previously existing cell, since all the cells of a plant have arisen from the multiplication by division of a single cell. Accordingly it is well at this point to observe cells in the process of division. For this purpose nothing is better than an examination of permanently mounted sections of the onion root-tip which has been fixed, imbedded, sectioned and stained (see Appendix for steps).

A careful study of a few such sections is sure to reveal cells in various stages of division. Cell division is essentially a division of the nucleus, which is quite complex. It is observed first of all that in cells about to divide the contents of the nucleus (nuclear reticulum) have become transformed into a thick winding thread. Next, the thread splits lengthwise, and then breaks up into rod-shaped pieces, each of which consists of two halves lengthwise, and is known as a *chromosome*. The membrane of the nucleus now disappears, and threads arise in the cytoplasm, which converge to opposite polar points and form the so-called nuclear spindle. Some of the threads extend uninterruptedly from pole to pole, while others become fastened to the chromosomes. The latter line up in an equatorial plane, half-way between the poles, and then one-half of each chromosome passes to one pole, the other half to the opposite pole, where they form a nuclear thread. This thread soon rounds itself off into a nucleus. The connecting fibres extending between the poles bulge out more and more, and new ones form between them. They thicken in the equatorial area, and the thickenings fuse together, forming a closed membrane called the *cell plate*. In its median plane a cell-wall is organized, dividing the mother cell into two. The fibres disappear and each new cell with its nucleus rapidly increases in size.

The process just described is known as *mitosis*, or *karyokinesis;* it is the *indirect* way of nuclear division or reproduction. This process is always followed in the formation of new cells. There is also a *direct nuclear* division, also called fragmentation, in which the nucleus becomes constricted and finally divides into two parts, which need not necessarily be equal in size. It usually occurs in old cells, and is not followed by cell division, but by a disintegration of the cell contents.

SUMMARY OF STRUCTURE OF A CELL.

- Cell
  - Cell Wall—Cellulose
  - Protoplasm
    - Nucleus and Nucleolus
    - Cytoplasm
    - Plastids
      - Leucoplastids
      - Chloroplastids
      - Chromoplastids
  - Vacuoles—Cell Sap

CHAPTER X.

EXERCISE IN SECTION CUTTING AND THE MAKING OF A PERMANENT STAINED MOUNT.

It is well at this point that the student be introduced into the art of section cutting and the making of stained permanent mounts (see also Appendix). He should be given opportunity to cut sections free-hand and with the microtome. For this purpose, practice under the eye and directions of an instructor is worth much more than many words in print, and therefore directions for cutting sections are omitted here; they are given in the Appendix. Mounting a section permanently simply means enclosing it within a medium in which it may be preserved indefinitely. Success in staining and mounting sections requires practice just as cutting them does. Sections selected for mounting should be such as can be used in later lessons.

For this lesson, mounts may be made of cross sections of the stem of Geranium (Pelargonium zonale), of Aristolochia (Aristolochia Sipho), and of the Corn stem.

For best results in the case of Geranium and Corn, material that has been fixed and imbedded in celloidin should be used. Good sections of Aristolochia can be made without imbedding, and material that has been preserved in 4 per cent. solution of formaldehyde is suitable for use. The sections should be 15 to 20 microns thick.

After the sections are cut, they are ready for the staining. Sections are stained not only for the sake of making more or less beautiful objects, but mainly for the purpose of differentiating the various kinds of tissues. Some tissues are so transparent that unless stained they would be quite invisible when mounted.

Single Staining.—Stain one or two sections of Aristolochia in Delafield's hæmatoxylin alone, and mount them in balsam, which is one of the best mounting media. The procedure consists of the following steps:

As the Aristolochia has been preserved in formaldehyde solution, all traces of the latter are first of all washed out of the sections by several changes of clean water. This is done by removing with a pipette the water in which the sections have been lying a few minutes, and replacing it with fresh water. After several washings, the sections are then passed

1 into 35% alcohol, 3 to 5 minutes.

2 into 50% alcohol, 3 to 5 minutes.

3 into Delafield's hæmatoxylin, 3 to 5 minutes.

4 into 50% alcohol, using 2 or 3 changes to remove all excess of stain.

Now examine the sections on a slide through the microscope (low power), and if they are stained properly, pass on to step 5. But if the stain is too deep, pass the sections into acidified alcohol (see Appendix) just for a few seconds, and then back into 50% alcohol; examine again as before and if the color is still too deep, repeat the treatment with acidified alcohol, which requires caution. When this process is properly carried out, it gives a most beautiful differentiation of color. All acid must finally be removed by washing the sections in several changes of 50% alcohol. They are then passed

5 into 70% alcohol, 3 to 5 minutes.

6 into 85% alcohol, 3 to 5 minutes.

7 into 95% alcohol, 3 to 5 minutes.

8 into oil of cloves, 5 minutes or until cleared.

9 mount in a drop of balsam, after draining off excess of oil from the sections.

Explanations.—The sections are not passed from water directly into the stain, because Delafield's hæmatoxylin is equivalent to about 50% alcoholic solution, and to pass delicate sections suddenly from water into such a liquid is likely to cause more or less distortion of cells, due to plasmolysis. To avoid this, the sections are passed first through graded alcohol (steps 1 and 2). For most plant tissues a change from water to 35% alcohol will do no harm, but for very delicate tissues (Spirogyra for example) this would be too sudden and a more gradual change would be required. For a similar reason, the sections must be passed through graded alcohols to reach the 95% alcohol (steps 5, 6, 7). The reason for treating the sections with 95% alcohol is because they cannot be mounted directly from water or from more or less aqueous media into balsam. Water and balsam do not mix. The sections must be dehydrated (freed from water) and alcohol is one of the best agents for this purpose. From alcohol, the sections are passed into oil of cloves for two reasons; first of all, because they cannot be mounted directly from alcohol into balsam, since these substances do not mix, and therefore some medium must be selected which will displace the alcohol and at the same time is miscible with balsam, and oil of cloves fulfills this requirement excellently. Secondly, oil of cloves serves as a clearing agent, that is, it makes the cell walls more or less transparent, so that light rays readily pass through them. Finally, balsam is used, it soon hardens, and the section is permanently enclosed.

Double or Differential Staining.—Take several sections ot Aristolochia that have been washed free of formaldehyde, and double stain them with fuchsin and hæmatoxylin by the following procedure: Pass them

1 into fuchsin (1% aqueous solution), 3 to 5 minutes.

2 into water, several changes, to free them from excess of stain.

3 into 35% alcohol, 3 to 5 minutes.

4 into 50% alcohol, 3 to 5 minutes.

5 into acidified alcohol, to remove all the color except from the more or less lignified tissues.

6 into 50% alcohol, several changes, to remove all traces of acid.

7 into Delafield's hæmatoxylin, 1 minute or less.

8 into 50% alcohol, several changes, to remove all excess of stain.

9 into 70% alcohol, 3 to 5 minutes.

Now examine, and if satisfactory, go on to step 10, but if too deeply stained, pass the sections into acidified alcohol for a few seconds, and then wash out the acid with several changes of 70% alcohol. Then pass

10 into 85% alcohol, 3 to 5 minutes.

11 into 95% alcohol, 3 to 5 minutes.

12 into oil of cloves, 5 minutes or until cleared.

13 mount in a drop of balsam, after draining off excess of the oil from the sections.

For sections imbedded in celloidin, see Appendix.

For contrast with the above double stain, stain several sections of Aristolochia, freed of formaldehyde, with fuchsin and Bismarck brown, following the previous procedure to step 6 inclusive. Then pass

7 into Bismarck brown (2% sol. in 70% alcohol), 1 minute or less.

8 into 70% alcohol, several changes, to remove excess of stain.

9 into 85% alcohol, 3 to 5 minutes.

10 into 95% alcohol, 3 to 5 minutes.

11 into oil of cloves, 5 minutes or until cleared.

12 mount in balsam.

Other stains may be used, but in double staining, care must be taken to select stains that offer contrast and that are truly selective. Fuchsin stains more or less lignified or cutinized tissues, hæmatoxylin or Bismarck brown stains those that are not thus modified. It would never do to select two stains that dye the same tissues.

CHAPTER XI.

TISSUES OF VARIOUSLY MODIFIED CELLS AS FOUND IN HIGHER PLANTS.

While it is true that all the essential phenomena which we call vital are manifested within the compass of a single cell, it is also true that the manifestation is feeble in comparison with that exhibited by cell aggregates, where there is division of labor among cells. All the higher plants are such aggregates of cells. They are made up of millions of them, and their life is not the mere aggregate of cells precisely alike, but rather that of sets of cells that have come to differ from each other in form and function, but all subserving the life of the whole organism.

These cell groups, which differ from each other in ways more or less important, but each of which is composed of similar cells, are called tissues. A tissue may, therefore, be defined as a group of united cells essentially alike, which have had a common origin and will perform a common function. There is a great variety of tissues, the individual cells of which differ more or less markedly from the typical cell already described.

It must be remembered, however, that all these tissues originate from a single cell, and that each cell of the mature plant, however great its deviation from the typical form, approximates the latter very closely in its early stages of development.

The various kinds of tissues are classified into four groups or series.

- I. Parenchymatous Series.
    1. Parenchyma; ordinary soft ground tissue.
    2. Collenchyma; thick-angled tissue.
    3. Sclerenchyma or Sclerotic parenchyma; stony tissue.
    4. Epidermal tissue.
    5. Endodermal tissue.
    6. Suberous or corky tissue.
- II. Prosenchymatous Series.
    1. Wood or libriform tissue.
    2. Tracheids or vasiform cells.
    3. Ducts or vascular tissue.
    4. Hard bast or bast-fibres.
- III. Sieve Series, including only sieve or cribriform tissue.
- IV. Laticiferous Series, including laticiferous or milk-tissue, which may be simple or complex.

Not all of the tissues are alive at maturity. Some are dead and merely serve as supporting tissues. All of the prosenchymatous series are of this nature, being devoid of protoplasm and mechanical in function. Likewise sclerotic and suberous tissues. Any one of the higher plants will contain most of the above tissues. If we examine stems of plants we find that the tissues are not arranged indiscriminately, but always follow a definite order. This order in Phanerogams is different from that in Cryptogams, in exogenous flowering plants it is different from that in endogenous ones. But in any particular case the order is always the same. *Exogens* are dicotyledonous plants and are characterized by having their stems sharply divided into bark and central wood cylinder, which latter is traversed by more or less numerous so-called medullary rays. *Endogens*, or monocotyledonous plants, have no sharply defined bark and wood core. Compare the section of Aristolochia, or Geranium with that of Corn, prepared in the preceding chapter.

To get an idea of the various kinds of tissues and the particular order of arrangement always met with in exogens, the stem of the common Geranium (Pelargonium zonale) serves very well. The stem of most any other exogen would answer, as the Bittersweet, Elder, Willow, Sycamore, Maple, Yellow-Parilla, Aristolochia etc., etc.

Use the mounted section of Geranium of the previous chapter, or make several thin cross-sections free-hand, or by the microtome, and mount one in a few drops of chlor-zinc-iodine solution. Examine with low power.

The first thing that strikes one is that the cells differ in size and shape, in compactness of arrangement, in thickness of cell-walls, in contents, besides that some stain blue while others stain brown.

Going from the exterior towards the center the very first layer of cells is the

1. *Epidermis* or external bounding tissue, a single tier of closely laid and similar cells, interspersed with hairs and having their outer walls thickened into a cuticle. If the stem be too old, the epidermis may not be present.

2. Beneath the epidermis are several tiers of tabular, brick-shaped cells in radial rows, the cells of the outer layers are empty and their walls stain brown, while the inner cells may contain protoplasm and the walls show some blue color. This is the *cork layer.*

3. *Collenchyma,* next to the cork, consisting of cells quite different in shape and arrangement from the cork cells, with cellulose walls, as shown by the blue color with chlor-zinc-iodine, rounded or polygonal and thickened at the angles where the cells join. The cells are rich in protoplasm. They are not as well developed nor as conspicuous in the Geranium as in some other plants.

4. The next layer of cells is *ordinary parenchyma* or *ground tissue,* a broad zone of large cells, with walls very thin and uniform and stained blue (cellulose), rich in protoplasm and starch contents, globular in shape, with small angular interspaces at the angles where the cells meet. The innermost cells of this layer form the *starch sheath* or *endodermis.*

All the tissues thus far described, beginning with the epidermis and ending with and including the starch sheath, are called collectively the *primary cortex,* while everything within the starch sheath is called the *stele.*

5. *Bast fibres,* next to the parenchyma, a zone of much smaller, angular, very thick-walled cells, stained a deep brown (lignified walls), compactly arranged and free from protoplasmic and starchy contents. The bast fibres are dead and act only as mechanical tissue. The cell-walls are stained brown like those of the cork cells, which might indicate that they are composed of the same material. That this is not so may be shown by adding to a fresh section a drop of phloroglucin reagent, followed after a time by strong hydrochloric acid. The cork cells remain unchanged, while the bast cells assume a fine reddish-purple color. The chemical substance composing the bast cells is known as lignin. This layer of cells is called the *pericycle.*

The fibrous nature of the cells is seen only in longitudinal section.

6. The next layer is a not very broad zone of small-celled tissue with blue stained walls. Under high power this is com-

posed of two sub-layers; the outer one consists of larger cells, more rounded, of *unequal* size, irregularly arranged and made up of two kinds of thin-walled cells, sieve tissue and a variety of parenchyma. These constitute the so-called *soft bast,* or *phloem.*

7. The inner layer of the two sub-layers is composed of very small cells, rich in protoplasm, very closely packed, in radial rows. These are primary meristem cells and form the so-called *cambium zone* of the stem. It is in this zone where growth takes place most actively, by which the stem continues to increase in diameter. When the bark is stripped from a stem the rupture takes place in this succulent fragile zone.

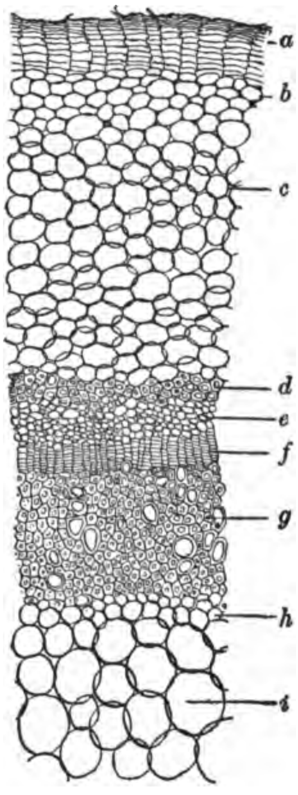


Fig. 38.—Geranium stem, cross-section. a, cork cells; b, collenchyma; c, parenchyma of middle bark; d, bast fibres; e. soft bast; f, cambium zone; g, ring of mixed wood fibres and vessels; h, i, small and large parenchyma cells of pith (Bastin)

8. Next the cambium is a layer more or less broad, according to the age of the stem, the so-called *xylem,* composed of cells for the most part like the bast fibres (see 5) and stained brown. These are *wood* or *libriform fibres* (lignified). Scattered mong them are other cells of larger diameter whose walls are also thickened and stained brown. These constitute the *vasiform* or *vascular tissue,* composed of *ducts* and *tracheids* of various kinds. Their function is to give strength and to convey the crude sap, i. e., water with its dissolved salts. All these cell-walls are lignified and behave like the bast fibres towards phloroglucin stain.

9. Interior to the wood circle is the *pith,* composed of large-celled parenchyma, with large inter-cellular spaces and starchy contents. The cells on the exterior are smaller than those towards the center and more compactly arranged. Many of the cells of the pith and the outer parenchyma layer contain dark masses, which, under the high power, are seen to be stellate crystals.

Make a longitudinal radial section and examine as before. The cork cells look about the same as in cross-section; the collenchyma cells are elongated; the bast fibres, wood cells and ducts are ***very much*** elongated and oblique-ended or tapering.

In the Geranium the differentiation of that portion of the stele between the pericycle and the pith into fibro-vascular bundles and medullary rays is not very clear. To observe this differentiation, examine a cross section of a one-year-old Aristolochia stem. In this no difficulty will be experienced in noting most of the tissues already mentioned and their differentiation into epidermis, primary cortex, starch sheath, pericycle, fibro-vascular bundles, medullary rays and pith. Observe the cambium ring. That portion of the cambium which separates the xylem and phloem portions of the bundles is called the *fascicular* cambium, that portion which crosses the medullary rays is the ***interfascicular*** cambium.

Examine cross-sections of other exogenous plants.

If permanent mounts of the Geranium or Aristolochia have not yet been made, make them now.

CHAPTER XII.

PARENCHYMA TISSUE.

In the parenchymatous series of tissues the cells are less modified, at least in shape, from very young cells than those of other tissues. They mostly retain to maturity the characteristics of the living cell, namely, protoplasm and nucleus and the power to form new cells by division. In some cases they become elongated and somewhat fibrous, but more commonly they are not much longer than broad, and have their ends square or rounded rather than oblique or tapering. In most of the tissues of the parenchymatous series the cells have thin walls, but in some instances the cell-walls are thickened by cellulose, cutinous or ligneous deposits.

Ordinary parenchyma or *soft ground tissue* is the least modified and most abundant of all the plant tissues. The walls are thin and frequently, though not always, composed of unmodified cellulose, commonly spheroidal or polyhedral in form, and the longitudinal rarely exceeds the transverse diameter. It includes most of the soft tissues of plants, such as the green cells of the leaf, the cells of pith, a considerable portion of the cells of bark, etc. Sometimes the cell-walls are unequally thickened, so as to present the appearance of markings of various kinds; indeed, they are seldom of uniform thickness, but commonly their membranous character and transparency make them appear so.

For the study of ordinary parenchyma cells the young Geranium stem, studied in Chapter XI, would serve very well, but for variety, sections from the soft green parts of other plants may be taken, such as the petiole of the Begonia, the Pumpkin stem, etc.

Let cross-sections be examined first in water, then stained with iodine and chlor-zinc-iodine.

The description of the parts of a typical cell given in Chapter IX answers very well for parenchyma cells. A structure not noted there is present in all green parenchyma cells, namely, small rounded granules stained deep brown, the *chloroplastids* or *chlorophyll bodies,* also often called *chloroplasts.* In the fresh unstained cell these are green. The chlorophyll granules give the green color to leaves, etc.

Another difference is the presence of small intercellular spaces between the cells, not found in the onion epidermis or any epidermis in fact. Starch grains also are often present, which assume a deep blue color with iodine, appearing almost black. Under high power the walls that have been stained blue by chlor-zinc-iodine appear finely punctate with nearly colorless dots. These are thin places or pits in the walls. Unlike the epidermal cells of the onion, parenchyma cells are nearly always globular, as may be seen from the cross and longitudinal sections of the Geranium stem (Fig. 38).

Parenchyma cells are not always globular and closely packed, but occur at times in modifications, both as to shape as well as arrangement. These modified forms are—

Stellate, or star-shaped cells

Folded—the cell-walls have internal folds.

Spongy—the cells are very loosely arranged.

Palisade—the cells are elongated and arranged like posts.

Pitted—the cell-walls marked by thin places of various dimensions and shapes.

Good examples of stellate cells can be studied in various species of Juncus. Some quite different in structure are also found in the petiole of the Spatterdock (Nymphæa advena). Examine a cross section of either of these.

Folded cells will be seen later in pine needles, spongy and palisade cells in leaves. For pitted cells, several plants might be chosen, but a convenient one is the plant known as the Sago Palm (Cycas revoluta).

Examine a cross-section of the petiole unstained in water. Under high power thick-walled rounded parenchyma cells will be found with a number of transparent, rounded areas looking like holes in the wall. These are thin portions of the otherwise thickened walls and not holes. On the edges the pits cause a beaded appearance (Fig. 39). Phloroglucin shows that the walls are somewhat lignified, giving a red color.

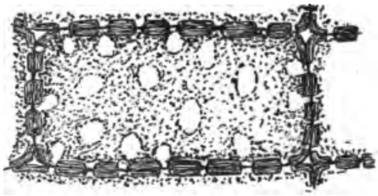


Fig. 39.—Pitted parenchyma cells.

Beautiful permanent mounts may be made by staining in aqueous fuchsin solution, passing the section through 70 per cent., 90 per cent. and absolute alcohol, then into eosin oil of cloves for ten or fifteen minutes and finally mounting in balsam. The thin areas of the wall will be stained pink by eosin and the thicker portions red by fuchsin.

CHAPTER XIII.

COLLENCHYMA CELLS.

Collenchyma, or thick-angled tissue, is closely related to ordinary parenchyma, but the cells are more elongated, often five or six times longer than broad, prismatic in shape and thickened at the angles. The thickenings are usually not lignified and the cells contain protoplasm, a nucleus and more or less chlorophyll. They are never found elsewhere than in close proximity to the epidermis or rarely in a similar relation to endodermal tissue, and one of the uses of the cells is evidently that of giving strength to the epidermis. Sometimes collenchyma forms a continuous circle, as in the petiole of Begonia and Grape; at other times it forms longitudinal bands, as in the petiole of Burdock and in the stem of Yellow Dock and Cow-parsnip.

The thickenings of the cell-walls in some plants are excessive and strongly diminish the cell lumen; in others they are slight. Sometimes they are confined to the angles, while at others they extend to a less degree to the rest of the cell-wall.

The petioles of Burdock, Begonia or any species of Grape afford good examples for the study of collenchyma.

The petiole of Burdock is bluntly angular, and seven ribs, darker in appearance than the rest of the surface, may be seen running lengthwise and forming the angles. These are the ribs of collenchyma cells. Make cros-sections and examine first in water with low and high power. The cells occur in patches just beneath the epidermis at the angles of the section. The cell-walls are much thickened, especially at the corners, and are strongly glistening (refractive), in this respect showing a marked contrast to the neighboring larger parenchyma cells. The cavities of the cells look dark by contrast with the shining cell-walls. At the border between collenchyma and parenchyma tissue the first line of cells of the latter tissue have one-half of their walls thickened, while the other half is thin.

The walls of the collenchyma cells are marked with delicate stratification lines. Such lines are common to thick walls generally and are due to the different layers containing different amounts of water, as may be proved by immersing in strong alcohol, which removes all the water, when the lines will disappear. Collenchyma is absent in most monocotyledonous plants. In longitudinal section the cells present quite a different appearance. The thickenings appear as long narrow bands, the cells are elongated, some being twice, others five to six times as long as broad, and blunt-ended. In some plants collenchyma tends strongly to fibrous tissue, being very long and greatly thickened and taper-pointed.

A good way to get a longitudinal view of the cells is to strip off the epidermis from a Lily petiole; the collenchyma cells will come off with it and may readily be studied in water or iodine solution.

In iodine solution the collenchyma cells are seen to contain protoplasm and a nucleus. If the excess of iodine solution be removed and sulphuric acid reagent be added, the cell-walls will swell and assume a blue color, which shows that they are cellulose in nature. Chlor-zinc-iodine solution gives the same result.

While studying collenchyma in the petiole of Burdock attention should be given to the whole section, as another illustration of the dicotyledonous type of stem structure. A number of distinct moccasin-like areas may be observed, arranged in a single circle and separated fron each other by plates of ordinary parenchyma cells. These are the *fibro-vascular bundles,* consisting of bast and wood fibres and tracheary vessels, just as in the

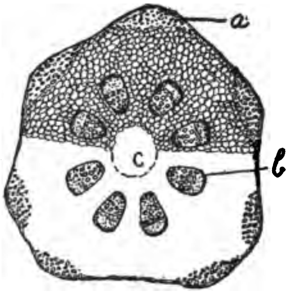


Fig. 40.—Sketch of cross-section of petiole of Burdock. a, patch of collenchyma; b, ring of bundles; c, pith with hole in center.

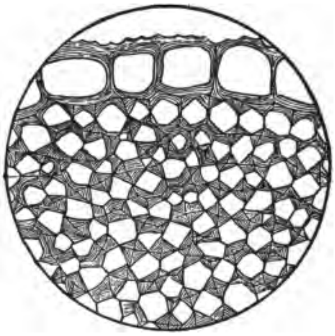


Fig. 41.—Cross-section of collenchyma of Burdock magnified, showing thickened angles of cell-walls (Bastin).

Geranium stem. But as in the case of succulent plants in general, so here the vascular bundles have not coalesced, so that there is no continuous ring of bast fibres, cambium zone and wood fibres and vessels, as is the case in the Geranium. Next to the circle of bundles comes the pith, the center of which is occupied by a cavity. Cork cells are absent.

Let a cross-section of Burdock soak a few minutes in phloroglucin solution, then add hydrochloric acid and note that the only lignified material occurs in the vascular bundles in the fibres and vessels.

CHAPTER XIV.

SCLERENCHYMA. SCLEROTIC CELLS.

The cells of this tissue are commonly called *stone* or *grit* cells. The cells differ from ordinary parenchyma ones in having the walls excessively thickened, so much so frequently that the cavity of the cell is nearly obliterated. Every gradation, however, may be observed between these and ordinary parenchyma. The walls of sclerotic cells are usually lignified and the thickening is deposited in layers, giving the appearance of concentric rings. These are the cells which give the great hardness to the outer coats of seeds and the shells of nuts. They constitute the gritty particles that occur in the flesh of some fruits, as the pear and apple, and are present in many barks, for example, Cinnamon, Oak, Viburnum, Cascara, etc. Stone cells are classed by some with parenchymatous tissues because of their origin and shape, but otherwise they have very little in common with parenchyma.

SCLEROTIC TISSUE OF WALNUT SHELL.

Use a piece of shell that has been softened by long soaking in 10 per cent. alkali and washed in acidified water. Cut thin sections in various directions, taking care not to let the razor run too deep. Mount in water and examine with low power, picking out the thinnest edge.

There will be seen a mass of rounded, somewhat polyhedral cells, pressed so closely together that no intercellular spaces are visible. The walls are extremely thickened and contain minute dots, as well as radial lines connecting the small cavity or lumen of the cell with the middle lamella. In this section the cells are almost colorless.

Put on high power. The radial lines can now be seen to be tubes, and the dots are tubes cut cross-wise, the ends appearing as dots. The tubes are known as *pore-canals* and are analogous to the pits in ordinary parenchyma cells. They probably serve to help the circulation of nutritive fluids from one cell to another, as is evidenced by the fact that the tubes of neighboring cells end opposite each other.

With careful examination the walls are seen to be made up of concentric layers. These are made more distinct by adding a drop of chloral-hydrate solution (5 chloral-hydrate to 2 water) and watching closely its swelling action. The lines come out plainly at first, but after a time disappear, owing to continued swelling.

Examining sections cut in various directions from the shell, it will be found that the cells have the same general appearance, showing that they are, in form, essentially like parenchyma cells, the difference being that the walls are immensely thickened in stone cells, and they no longer take part in the vital processes of the plant, but act as mechanical tissue; in fact, in some cases they are elongated and fusiform, so that it is difficult to distinguish them from prosenchyma fibres. All gradations are found between parenchyma and stone cells and between stone cells and bast fibres.

Add phloroglucin and hydrochloric acid to a section. The walls are stained red, proving lignified materiali.

Stone cells may be studied to better advantage by isolating them by Schulze's maceration solution as directed under this reagent (see Appendix). Stain a section treated thus with methyl-green and mount in water, and tap the cover glass with a teasing needle, when the cells will separate in virtue of the middle lamellas, which unite the cells, being dissolved away by the maceration fluid.

Examine sections cut in various directions from cocoanut shell. These cells have a natural brownish-yellow color, and their boundaries are more easily seen than in the case of the walnut cells. While many cells are almost circular in outline, others are somewhat elongated. This fact indicates that the cells tend towards the fibrous condition and run in different directions, so that in the same section some are seen in cross-section and appear round, while others are seen lengthwise and appear elongated. The cavities are filled with a dark material. In general structure the cells resemble those of the walnut shell. Ground cocoanut shells are used frequently as an adulterant of ground spices and should, therefore, command close study by the student.

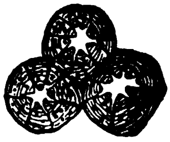


Fig. 42.—Stone cells from walnut shell.

Most excellent sections of the stony tissues of walnuts, peach stones, hickory nuts, etc., may be gotten by the following method: Saw off as thin a slice as possible with a hack saw. Rub it between two pieces of fine sandpaper until it is ½ mm. or less in thickness, then rub it between two smooth hones, keeping the latter wet with water When the section is thin enough, wash it well in water, using a pipette. It is now ready for study. If the section is to be permanently mounted, dehydrate it in 95 per cent. and absolute alcohol, clear in oil of cloves, and mount in balsam. The section, unless very thin, need not be stained. The above process is rather slow, but yields fine sections.

To study the stone cells as they occur in barks, wrap pieces of the bark of Cassia Cinnamon and Cascara in wet filter paper and keep in a closed bottle until they are soft enough for sectioning. Also immerse a piece of Viburnum (stem) bark in dilute (1 or 2 per cent.) alkali until soft, and finally wash thoroughly in water. Cut cross-sections between pith or cork and mount in water or chloral solution. Cinnamon has an interrupted chain of colorless stone cells running tangentially about midway of the section. Cascara presents large masses of yellow stone cells at about one-third the width of the section from the outer edge. Viburnum has similar masses of stone cells distributed over the whole section. The stone cells can easily be recognized by their structure as well as by the red stain they assume with phloroglucin and hydrochloric acid.

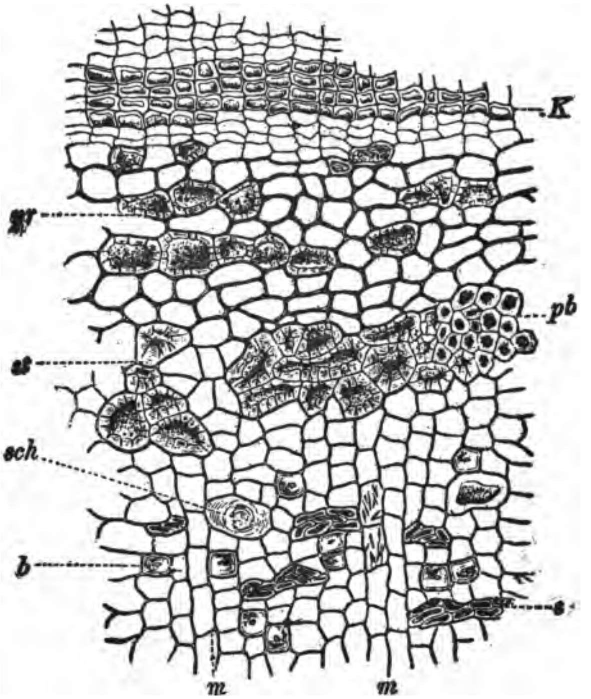


Fig. 43.—Cassia bark, cross-section. K, stony cork cells; pr, cortical parenchyma, with stony parenchyma cells; st, stone cells forming an interrupted ring; b, bast fibre; pb, a group of primary bast fibres; sch, secretion cell; m, medullary rays; s, sieve tubes (Moeller)

CHAPTER XV.

EPIDERMAL TISSUE.

This tissue has already been met with in the exercise on the onion epidermis, but the latter is not quite a typical example, as there are no *stomata* or *breathing pores* present, which are always found when an epidermis is exposed to the air. Since the onion scale epidermis is not exposed to the air there is no need for the pores and they are consequently absent. In other respects the epidermis of other plants resembles very much that of the onion.

This tissue constitutes the primary covering of the plant. It usually consists of one, but sometimes of two or three layers of cells. The cells are closely packed together, leaving no intercellular space except the breathing pores, and commonly they have that portion of the cell-wall which faces exteriorly considerably thickened and cutinized and are usually flattened. When seen in surface view they often appear sinuous or irregular in outline, but sometimes they are straight-sided and regular. In many plants they are somewhat elongated in the direction of the length of the organ, especially in the cells on the veins on the under surface of leaves.

The cells are usually quite clear and evidently serve as reservoirs for surplus water. The protoplasm lines the cell-wall as a very thin film. The different parts that were noted in the onion epidermis are noticed just as plainly in other cases. In most plants, notwithstanding their exposure to the light, there are no chlorophyll bodies present in the epidermal cells, except in the guard cells of the stomata. Ferns are exceptions to this, likewise some plants, particularly monocotyledons, of shady habitats.

STOMATA OR BREATHING PORES.

These pores are minute apertures, usually surrounded by a pair of crescent-shaped cells called *guard cells.* These are much smaller than the epidermal cells and are much richer in protein matters, containing a nucleus, protoplasm, numerous chlorophyll bodies and occasionally oil globules.

By means of the pores the plant transpires the superfluous water taken in by the roots and allows the excess of oxygen not used by it to pass out, but their chief use is to allow the carbon dioxide necessary for the plant's life to enter. They always open into a large intercellular space, the substomal cavity. Thus the outside air is in free communication with the whole interior of the plant stem and leaves, since the air circulates freely through the intercellular spaces which are in communication.

Communication with the interior of the plant takes place only through the pores, since the cutinized exterior of the surface of the epidermal cells is highly impregnable to water and air. Hence epidermis is an excellent protection against evaporation of moisture from the interior of the plant.

The size of the breathing pore is regulated by the guard cells. It is found that the outer and inner faces of the guard cells are thickened and *cutinized*, while the radial walls in close contact with the surrounding epidermal cells, are *not cutinized* and are very thin.

Now when the cells are turgid (filled with water), and the sun is shining—two conditions necessary to the manufacture of food by the chloroplastids in leaves (see Chapter XXV on Leaves)—the stomata stand open, while in darkness and when turgidity is low in the leaves, they close. Their action is explained as follows:—The guard cells contain chloroplastids which manufacture sugar, etc., when the sun is shining. To do this, they call upon the surrounding cells for water, becoming thereby turgid and swollen, and, in this condition, draw apart and leave a passage between them into the interior of the leaf. This action is due to the fact that the radial wall, the one farthest from the opening, is more extensible than the wall bordering the opening, and bulges out farther with the swelling of the cell, and drags the wall bordering the opening with it. A mechanical device can be constructed that illustrates very well the action of guard cells, thus:—Into a two-hole rubber stopper that will fit a faucet, fix two pieces of glass tubing about two inches long, and fasten to the latter two pieces of black rubber tubing about one and a half inches long. Plug up the free ends of the rubber tubes and tie them together. Then, with a very sharp knife, pare rather thin the outer side of each piece of rubber tubing, attach the stopper to a faucet and cautiously turn on the water. The thin outer sides of the rubber bulge out, and the pieces draw apart, quite similar to the guard cells.

In some cases epidermis is smooth, but in the majority of cases it is roughened by hairs or glands and the walls are wavy in outline. The number and distribution of stomata varies greatly in different epidermal tissues. They are found principally on the under surface of leaves. In some leaves many are found on the upper surface, in others none at all are found.

Hold a leaf of the common trailing garden plant, known popularly as the Wandering Jew, against the side of a large cork, and with a sharp razor cut off tangentially little patches of the epidermis, both from the upper and lower surfaces. Some of the underlying green cells may be taken with the epidermis, but the edges of the sections will generally consist of epidermis alone. A few of the green cells, in fact, are desirable, as they will afford an opportunity to study incidentally chlorophyll granules.

Mount a section from the upper surface in water and examine with low and high powers. The epidermal cells are hexagonal in shape, transparent, free from chlorophyll granules and have no intercellular spaces between them. With iodine solution the protoplasm stains yellow and is more readily seen. In all essentials the cells are like those of the onion epidermis. Note that stomata *are absent.*

In the same manner examine a section from the lower surface of the leaf. The epidermal cells are pretty much the same in appearance, but scattered among them are numerous stomata, easily distinguished by the pairs of crescentic cells with dense contents and chlorophyll granules. These cells guard an opening or pore which is readily seen. With iodine solution the granules in the guard cells are very deeply stained.

Note the relative sizes of epidermal cells and guard cells and whether the latter all point in one direction or not.

In some of the sections in which the underlying green parenchyma was cut with the epidermis there may be seen long strands of spirally-marked tubular cells. These are the spiral wood vessels of a small vein of the leaf. This kind of vascular tissue will be studied in a later exercise.

Examine the epidermis from both sides of a leaf of the Cultivated Lily and compare with that of the Wandering Jew. Note the absence of plant hairs from both leaves.

Mount fragments of a Senna leaf in a few drops of chloral hydrate solution, one with the upper surface turned up, one with the lower surface turned up and cover with a glass. Heat gently till the liquid begins to boil and keep it at that temperature for a few moments, then cool, add another drop of liquid if necessary and examine.

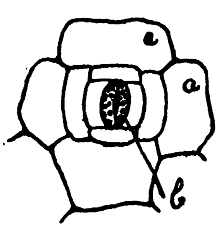


Fig. 44.—Epidermis of Wandering Jew. a, epidermal cells; b, guard cells of a stoma, with chlorophyll granules.

The leaf has become sufficiently transparent that the epidermal cells, stomata and hairs can be distinctly observed. The epidermal cells are polygonal in shape, stomata are present on both surfaces and the hairs are one-celled with thick, warty walls, often curved.

Often the epidermis can be separated by warming a fragment of a leaf in a solution of potassium hydroxide (about 2 per cent.) until it boils, cooling and pressing the cover glass firmly, while at the same time giving it a sliding motion. This method may be tried when chloral hydrate solution fails to clarify sufficiently.

Cross-sections of stomata will be examined in the exercises on stems and leaves.

CHAPTER XVI.

EPIDERMAL APPENDAGES.

All the outgrowths or appendages of the surface of a plant are known as *trichomes,* which means literally *hairs.* They consist of one or more cells usually arranged in a row or column, sometimes in a mass. They may be formed solely by epidermal cells, or, as in the case of the so-called *emergences,* cells lying more or less deeply in the sub-epidermal tissue may also take part in their formation. The most common forms of trichomes are—

1. Hairs—these are the principal form; they are outgrowths from some of the epidermal cells.
2. Bristles—a single pointed cell, much thickened and hardened by deposits of a calcium salt or silica in the cell-wall.
3. Prickles, one form of the so-called emergences.
4. Scales, more or less flattened trichomes.
5. Glands—generally short, bearing one or more secreting cells. They may originate either as hairs or as emergences.
6. Root-hairs, long, thin, single-celled and subterranean.
7. Sporangia of ferns.
8. Ovules of flowering plants.

Trichomes originate mostly from the growth of single epidermal cells, and on their first appearance consist of slightly enlarged and protruding cells. These may elongate and form single-celled hairs, which may be simple or variously branched. The simplest of hairs are the papillæ, which are epidermal cells, whose external wall protrudes more or less conically. Possibly the most important of plant hairs are those which clothe so abundantly the young roots of most of the higher plants and to which the name of *Root-hairs* has been applied. These are single cells which have very thin and delicate walls, and are the active agents in the absorption of soil water with its dissolved salts. On the above-ground parts the hairs frequently have the terminal cell developed into a secreting cell, carrying gummy, resinous or other products. Such trichomes are known as *glandular hairs.* The functions of trichomes are as various as their shapes.

A good example of simple hairs, which is familiar to every one, is the cotton of commerce. Cotton consists of the hairs on the seeds of the cotton plant. They have been studied in Chapter II.

HAIRS AND GLANDS AS FOUND ON A GERANIUM.

Make cross-sections of the young stem of the common Geranium, which need not necessarily be very thin. Mount several in water and examine the circumference of the sections under low power. Two kinds of hairs will be seen, simple and glandular.

Under high power the simple hairs are very long, consisting of a row of tapering cells which contain transparent protoplasm and nucleus, easily seen by applying iodine solution. The end cell is long and pointed. The hair fits in among the epidermal cells. The cell-walls are somewhat thick and the cross partitions are well marked.

There are three kinds of gland hairs.

One kind is rather long, consisting of a stalk of about six cells (count the actual number), terminated by a larger gland cell, which is round and full of contents. The two or three cells just below the gland cell are suddenly narrowed. On the top of the gland cell there is a crescent-shaped glistening mass which is found to lie in the cell-wall itself between the outer and inner layers. This mass dissolves on applying alcohol or ether, which indicates that it is probably resinous. Additional evidence of this is given by the reaction with alcannin solution, which stains the mass red. The resinous matter is secreted by the gland cell and forced out into the cell-wall where it accumulates. Iodine solution shows the presence of protoplasm and nuclei in the cells.

Soak some sections 20 to 30 minutes in strong alcohol, also in alcannin solution and note the effect upon the resinous matter.

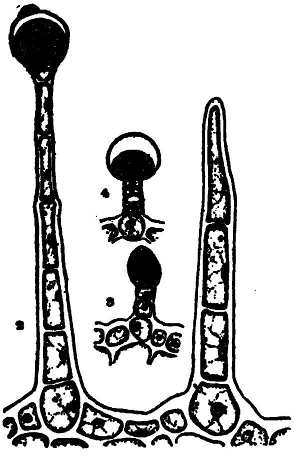


Fig. 45.—Hairs on Geranium stem. 1, simple hair; 2, 3, 4, three forms of gland hairs (reduced, from Bastin).

The other two kinds of gland-hairs are very short. One has an oblong gland while the gland cell of the other is round. Note the number of cells in the stalk of each. The cells are very short.

Note which of the three kinds is most abundant.

Submit a section to the action of chlor-zinc-iodine solution for about half an hour and then examine. The outer layer of the walls of the hair-cells is stained brown, showing cutinization; the inner portion of the walls may be blue, showing cellulose substance.

Try the clearing effect of chloral-hydrate solution on the gland-hairs.

The contents will gradually disappear, leaving the cell-wall distinct, and the gland-cell structure may now be studied.

It is thought that the purpose of hairs and glands is to afford protection to the plant against insects, etc. The odor of the Geranium is due to the secretions of the gland-hairs.

HAIRS ON OTHER PLANTS.

For variety, let the hairs on the following plants be studied:

Clamp a piece of Mullein leaf btween pith and cut several sections. Mount them in water and examine. A dense growth of branched hairs covers the leaf and beneath these, close to the surface, will be found a few short glandular hair.

Clamp a moderately large vein (with a portion of the leaf on each side) of Stramonium, Digitalis, Belladonna and Hyoscyamus leaf respectively in pith and cut cross-sections. Examine these in water or chloral hydrate solution. Compare the hairs and make drawings. These are leaves of four important official drugs and the comparison of the hairs should therefore be of special interest.

CHAPTER XVII.

STARCHES.

The green color of the leaves of plants is due to a colored substance called chlorophyll, which is diffused through certain protein granules, the plastids, in the cells. The function of this substance is to utilize the energy of the sun's rays in converting carbon dioxide and water, the main food materials of plants, into some form of carbohydrate. The decomposition of carbon dioxide and water by the action of light, and the combination of the products into glucose and starch, is termed ***photosynthesis*** (see Work of the Leaf, Chapter XXV). As there outlined, the first visible product of photosynthesis is the starch formed in the chloroplastids. Starch, however, appears in the chloroplastids only when the glucose, which is the first product formed, is manufactured more rapidly than it is removed from the leaf (as always occurs in bright day light). The glucose is removed during the day as fast as possible, and at night the surplus, which during the day was converted into starch, is again transformed into glucose and also removed. Most of it finds its way to places where growth is going on, but the surplus is stored away in various parts of the plant, as reserve food.

There are various reserve food materials found in the plant, and starch is one of the most important. It is found in various parts of the plant, for example, the stems of certain palms, which are gorged with it; it is the principal substance in many tap-roots, root-stocks, corms, bulbs, tubers; many fruits and seeds, as grains, pulses, bananas.

The method by which starch is stored is, in all cases, the same. It is accomplished by the leucoplastids, called also in this connection *amyloplastids*. These remove by chemical action a molecule of water from each molecule of glucose, and deposit the resulting starch in their own substance. This goes on rapidly at night, and slowly, if at all, by day; as a result, the starch grains of storage tissues regularly show layers corrresponding to their periodic activity. The starch thus stored serves as food material for starting new growth and continuing it, until a supply of food by the new leaves can be depended upon.

The power of building up protoplasm form starch is possessed by all living cells, whether possessing chlorophyll or not, and independent of sunlight, but no *new* carbohydrate is ever formed without light. A tuber will sprout and grow in the dark until all the starch is used up, when growth ceases, and to renew growth it must be brought into light.

DESCRIPTION OF STARCH GRAINS.

They are hard and of various sizes and often possess shapes and markings sufficiently characteristic to identify the plant from which they come. They vary from 1 to 100 or even 200 microns. Starch gains are *simple* or *compound*. Compound grains consist of two or more simple grains united to form larger grains. Potato, wheat, arrow-root, corn and ginger starch are examples of simple grains, while oat, rice and colchicum starch are compound.

Nearly all starch grains possess a *nucleus* or *hilum*, around which the granule is built up in layers, which differ from each other in transparency, owing to different amounts of water in the different layers. The layers are concentric or eccentric according as the nucleus is central or placed to one side. Examples of these are bean and potato starch respectively.

Starch grains differ from one another in a number of ways, some of which are the following:

Simple or compound.

Size and shape of grains.

Position of hilum (central or eccentric).

Number and distinctness of stratification lines.

Degree to which the hilum is fissured.

Character of fissure.

POTATO STARCH.

To see the manner in which starch is packed in the cells of storage tissue, examine cross-sections of the potato tuber.

Cut an oblong block about a half-inch square on the end from a potato, having the outer coat on one side. Cut thin sections perpendicular to the coat and mount one in water. Examine first under low power and then under high power.

There will be seen on the exterior a layer of cork cells like rows of bricks. Next these are some small, closely packed parenchyma cells, rich in protoplasm, but containing few starch grains and these are small. There may also be seen cubical crystals which look like mineral crystals, but are protein, as they stain with iodine and are dissolved by caustic potash. Farther interior the cells are very large and filled with large-sized starch grains, and the cubical crystals are wanting.

Apply dilute iodine solution. The grains assume a dark blue color, the protoplasm stains brown and the cubes the same. It will be observed that the younger starch grains in the outer small parenchyma cells are mostly found in groups. Possibly it may be observed that the grains are grouped around a mass of protein granules, each one of which is attached to a starch grain. These protein granules are the starch-builders, the *amyloplastids*. They are similar to chlorophyll, and all starch grains are formed by one or the other kind of these bodies. As the starch grain grows, the amyloplastid is stretched thinner and thinner, until it becomes invisible. It never disappears, however.

Scrape a slice of potato with a knife and mount in water. The grains are ovate, with one end smaller than the other and a hilum or nucleus at the smaller end. The hilum is surrounded first by concentric lines which, farther away, become eccentric. This shows that at first the growth of the grain was equal on all sides, but afterwards became much greater on one side than on the other. This view is borne out by the fact that in young grains the hilum is central. Some of the lines or striations are more strongly developed than others, and some of the grains show scarcely any lines. Nearly all the grains are single, but some double grains may be found containing two nuclei, each with concentric and eccentric markings about it and a distinct dividing line. Some grains are not double, but contain two nuclei, i. e., are bi-nucleated.

The nucleus is usually a circular spot in the potato grain, but sometimes it is fissured. The small end of the grain, where the nucleus is found, is thicker than the broad end, the grain being shaped somewhat like a clam shell. To observe the exact shape of the grains they must be seen from all sides. This is readily done by pressing gently on the cover glass with a needle, when the grains will roll over and may be seen in various positions. Another way to cause the grains to roll is to place a drop of alcohol at the edge of the cover glass, which, by mixing with the water, causes currents. When thus observed, the grains are seen to be somewhat flattened (Fig. 46, II).

Let a drop of 5 per cent. caustic potash solution run under the cover glass and watch the starch grains as the alkali comes in contact with them. They swell, and at first the layers become more distinct, but after a while they grow less distinct and finally disappear. The more watery layers at first absorb water under the action of the potash more rapidly, and thus stand out more distinctly. Finally the whole grain dissolves and disappears. Other reagents which cause starch to swell and dissolve are concentrated solutions of chloral hydrate, zinc chloride, calcium chloride, strong hydrochloric and sulphuric acids, oil of cloves.

Mount some potato starch in water and apply a gentle heat near the edge of the cover glass until the grains there become translucent, then quickly remove the flame and cool the slide. Compare the grains that have been affected by heat with those that have just begun to change and those that are still intact. Heat causes the grains to swell and ultimately to assume a gelatinous form. Intermediate granular and translucent stages occur in those grains that have not been heated too strongly. The temperature of gelatinization varies appreciably with the kind of starch.

Examine potato starch mounted in glycerin, also in oil of cloves. The grains appear brighter and the lines are almost invisible. If an air globule be imprisoned in the hilum it will appear as a dark spot. Glycerin and oil of cloves and any similar liquids are unsuited as a medium for mounting starch because of their high refractive power, which causes details of structure to be almost invisible.

OTHER STARCHES.

MARANTA.—A large proportion of the edible starches obtained from the rhizomes or root-stocks of various plants are known in commerce under the name of arrow-root. Properly the name should be restricted to the starch yielded by two or three species of Maranta, the chief of which is Maranta arundinacea. According to the country from which it is derived, maranta starch is known as Germuda, St. Vincent, West Indian or Natal arrowroot. The grains are simple and mostly ovoid, the largest ones being marked by fine striations, but less distinct than those of potato starch. The nucleus is rounded, linear or star-shaped and usually placed eccentrically. The grains are rather large, but smaller than potato starch (Fig. 46, III).

TOUS-LES-MOIS, *Tulema* or *Queensland* arrow-root, is obtained from several species of canna, a genus closely allied to maranta. The grains resemble those of potato starch, but are much larger.

CURCUMA or *East Indian* arrow-root is obtained from the root-stocks of several species of the genus Curcuma (Zingiberaceæ) chiefly Curcuma angustifolia. The grains are ovate and taper to a nipple-like projection at one end, in which the hilum is located. The grains are simple, rather large and so flat that on edge they seem rod-shaped (Fig. 46, VIII). Ginger starch is shaped somewhat like that of Curcuma.

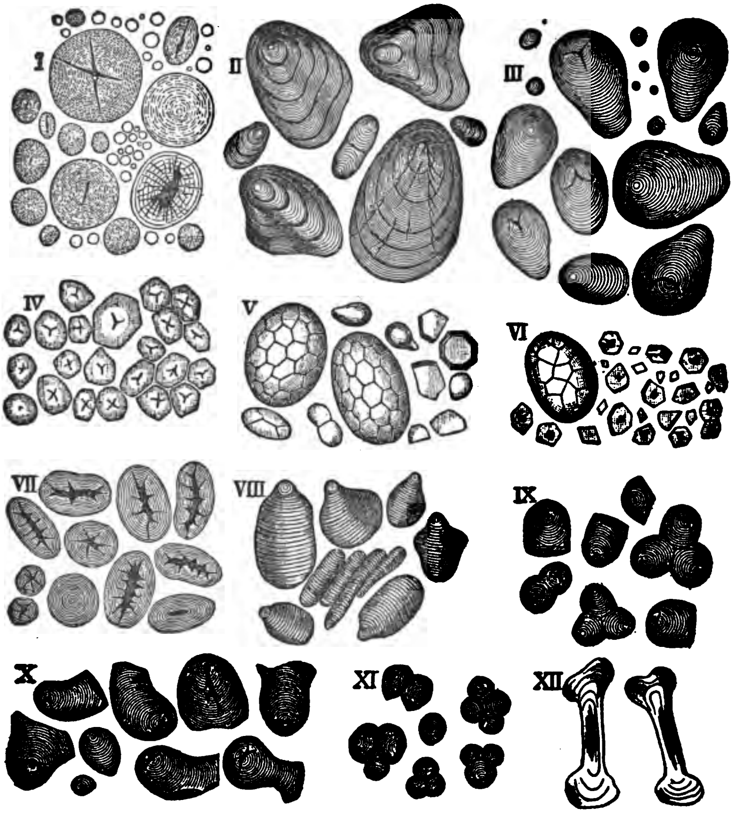


Fig. 46.—Starches.
I, Wheat; II, Potato; III, Arrow-root; IV, Corn; V, Oat; VI, Rice; VII, Bean; VIII, Curcuma; IX, Tapioca; X, Sago; XI, Sarsaparilla; XII, Euphorbia.

BRAZILIAN arrow-root, cassava, or tapioca of commerce, is manufactured from the starch obtained from the tubers of Manihot utilissima. Most of the grains are blurred, which is due to the heating in the process of manufacture, but many grains may be found uninjured. Soak some of the starch a few hours in water, mash a little out on a slide in water, cover and examine. The unchanged grains appear as in Fig. 46, IX; the others are more or less gelatinized.

BRITISH arrow-root is potato starch, which is sometimes sold under this name. The French excel in the preparation of imitations of the more costly starches from potato starch. Its chief use, however, as an edible starch, is for adulterating other more costly preparations. It can easily be distinguished under the microscope.

SAGO, or pearl sago, of commerce is obtained by heating and stirring the moist starch of the sago palm, Metroxylon Sagu. As in the case of tapioca, many of the grains are gelatinized, but some are still intact, which appear as in Fig. 46, X.

MAIZE or corn starch is obtained from the fruits of Zea Mays. The grains are simple, rounded or polygonal and tolerably uniform. The hilum appears as a point or more often as a stellate cleft, less frequently as a large central cavity. This starch is one of the most frequently used adulterants of powdered articles, and hence should be closely studied (Fig. 46, IV).

WHEAT starch is obtained from several species of Triticum. It consists of large rounded grains and numerous small ones. Hilum and striations are seldom visible. The grains are oval or concavo-convex in shape. Wheat starch is also used as adulterant (Fig. 46, I).

RICE starch comes from Oryza sativa, and may be examined after soaking rice grains for three hours or more in water. The grains are very small and angular, with no hilum. The small grains result from the breaking apart of larger compound oval grains, some of which may be present in the mount (Fig. 46, VI).

OAT starch from Avena sativa resembles rice starch very much. Some of the granules are rounded, semi-circular or lemon-shaped, which helps to distinguish it from rice starch (Fig. 46, V and VI).

CHAPTER XVIII.

ALEURONE GRAINS.

As stated in the preceding chapter, there are other reserve food materials found in the plant besides starch. There are two kinds of foods thus stored; one nitrogenous or protein, and the other non-nitrogenous. Of the latter class, starch, fats and oils are most common forms, glucose, lævulose and saccharose are less common, while cellulose, inulin, glucosides and mucilage are only occasionally found. Different kinds of proteins and amides are the chief forms of the nitrogenous class. Proteins are the most complex and most important of plant foods, resembling in their composition living protoplasm itself. There are many different kinds of proteins in plants, some occurring in solution in the cell sap, some as amorphous, very minute granules, and some as definite, rounded granular bodies, generally known as *aleurone grains*. These are found chiefly in seeds and most abundantly in oily or starchy ones. They are usually rounded granules, often very small, but sometimes quite large, as in Croton and Castor seeds and in the Brazil-nut. In some cases the granules appear homogeneous in structure, as in Peony, Almond, Cherry and Apple seeds; in other cases they contain various substances, as oily matters, mineral crystals and crystalloids, as in Brazil-nut, Pumpkin and Castor seeds, Walnut.

The ground substance of aleurone grains is dissolved or attacked by water, hence the latter is an unsuitable mounting medium. Alcohol, glycerin or fixed oils have no solvent action on the grains. Dilute alkalies readily dissolve the aleurone grains except the mineral matter in them.

CASTOR SEED.

Remove the hard seed-coat and make thin sections of the endosperm. Mount one in strong glycerin and examine with low and high powers. Not much will be made out with low power. The cells will be seen to be crowded with rounded granules, looking much like starch.

Under high power, rounded or ellipsoid bodies, imbedded in a finely granular matter, fill the cells. These are the aleurone grains. After a little time the clearing effect of the glycerin shows that the grains are not homogeneous, but contain a denser, polygonal body looking like a crystal and known as a *crystalloid* which varies in size, being often nearly as large as the grain. Lying alongside of this is seen a globular body, strongly refractive and composed of magnesium and calcium phosphates and known as a *globoid* (Fig. 47).

Add strong iodine solution to the section in glycerin. The grains stain brown, especially deep in the crystalloid, indicating the protein nature of the latter. The globoids remain unstained.

There is no blue coloration, showing the absence of starch.

Mount a fresh section in water and watch the aleurone grains. The ground substance soon swells and dissolves, leaving the crystalloid standing out more distinct. After a time this also swells and loses its angles and finally disappears. Oil globules may also be seen to collect and run out from the ruptured cells. These have a refractive appearance, being bounded by a dark band.

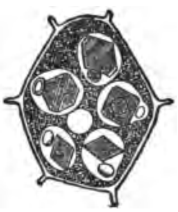


Fig. 47.—Aleurone grains in cell of castor seed, showing crystalloids and globoids (Sachs).

If fresh sections be mounted in alcannin or cyanin solution the oil globules will stain after a time a deep red or blue. Oil is a reserve food for the plant, like starch and aleurone, and is found in a great many seeds and spores, often in large quantity.

Mount a section in dilute alkali (about 1 per cent.) and watch a grain closely. All parts dissolve except the globoid.

CHAPTER XIX.

CHLOROPLASTIDS OR CHLOROPHYLL CORPUSCLES.

When speaking of starch, it was noted what importance these bodies are to the life of plants, and that without them plants could not exist. They are the most important structural form of protoplasm. They are the bodies which give the green color to plants and are commonly rounded, oblong or flattened in shape. An odd form is the spiral bands in Spirogyra (which see).

Chloroplastids are protein in nature, having the power of growth and division, and always closely associated with ordinary protoplasm, and are hence to be regarded as part of the living protoplasm.

In many plants chloroplastids are small and difficult to study, but in others they are easily studied. Most any Moss, the prothallia of Ferns, Eel- grass, Water-weed, are excellent objects of study.

Mount a few fresh leaves of a Moss in water and examine the cells lying near the edge of the leaf. The cells contain numerous rounded greenish bodies—the chlorophyll bodies. Note the closely-packed, rectangular-shaped, somewhat thick-walled cells. Try to observe chlorophyll bodies that are constricted in the middle. Such are in the act of division.

Run strong alcohol under the cover glass. The green bodies are slowly bleached, the chlorophyll is dissolved out and forms a greenish solution in the cells. From this experiment it is concluded that the bodies consist of two parts, a protein ground work, through which is diffused the chlorophyll or green colored substance.

Add iodine solution to a leaf that has been bleached with alcohol. The transparent protoplasm of the cells is now stained brown and made visible. The ground substance of the chloroplastids also stains brown, indicating a protein substance.

Starch grains in chloroplastids. Put some leaves of a vigorously-growing Moss that has been exposed to sunlight for two or three hours in alcohol until bleached. Then mount a leaf in water, focus it and apply a drop of chloral-hydrate iodine solution at the edge of the cover glass and watch closely the chlorophyll body as the reagent comes in contact with it. The ground substance is seen to swell rapidly and to become transparent, leaving the starch grains, stained blue, visible. The latter are small and elongated. By the continued action of the chloral the whole structure gradually disappears. The starch that is formed in the chlorophyll bodies in the daytime is dissolved during the night and transferred to other parts of the plant. (See Chapter XVII.)

CHAPTER XX.

SECRETION SACS, INTERCELLULAR AIR SPACES AND SECRETION RESERVOIRS.

Some cells at maturity lose their protoplasm and their proper cell character and become filled with secreted matters. These form the *secretion sacs*. They are of various forms, but more commonly resemble parenchyma cells in appearance and character of their walls. Sometimes they are much elongated and resemble latex tissue. The sacs are given names according to the secretion they contain, thus, resin sacs, mucilage sacs, etc.

Intercellular air spaces are more or less abundant in nearly all multicellular plants, their probable function being to supply air to the interior tissues for respiratory purposes. In aquatics the spaces are usually large and often regular in shape, while in most terrestrial plants they are small and angular. Air spaces are of two kinds, *schizogenous* and *lysigenous*. The former are formed by the splitting of the cell-wall common to two or three cells. The latter result from the breaking down of some cells, leaving a space. Secretion reservors or canals and intercellular air spaces differ from each other only in their contents, the former containing resins, gums, oleo-resins, etc., the latter only air.

INTERCELLULAR AIR SPACES AND RESIN SACS.

Calamus (Sweet Flag) Rhizome.—Make thin cross-sections of the fresh rhizome or of the dried one after having soaked it in water to soften it. Mount a section in water and examine the outer third of the section. The parenchyma cells are loosely arranged in chains with large and tolerably regular intercellular air spaces. Most of the cells are filled with starch grains, to-

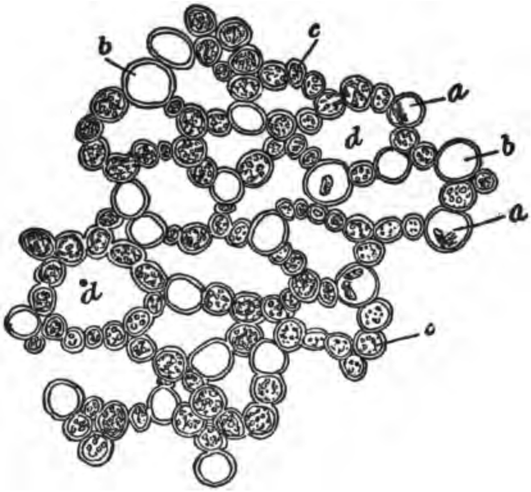


Fig. 48.—Part of cross-section of Calamus rhizome. a, volatile oil and resin sacs; b, sacs with transparent secretion; c, starchy parenchyma cells; d, air space (Bastin).

gether with protoplasm and nucleus, but scattered here and there among these are larger spherical cells filled with a refractive or glistening material, sometimes intermixed with a brownish solid. These are the secretion sacs (Fig. 48). The refractive contents of the sacs are not saponified by caustic potash, which fact indicates a volatile oil.

Further evidence in favor of this conclusion is given by applying cyanin solution to a section when after some time the contents are stained blue. The solid brown matter found in some of the cells is resin and also stains blue with cyanin.

Ginger Rhizome.—Cut cross-sections from the dry rhizome, mount a thin one in water and examine with low power first. The section is made up for the most part of large starch grains in ordinary parenchyma cells, but here and there may be seen groups of thick-walled light yellow cells, the fibres and vessels of the wood bundles. In addition to these there will be seen more or less distinctly lighter or darker yellowish homogeneous masses. They are the oleo-resinous contents of secretion sacs which are scattered over the whole section. If dilute iodine solution be added the section turns dark, but the groups of woody cells now stand out more conspicuously in contrast by their deep yellow walls and the resin sacs also.

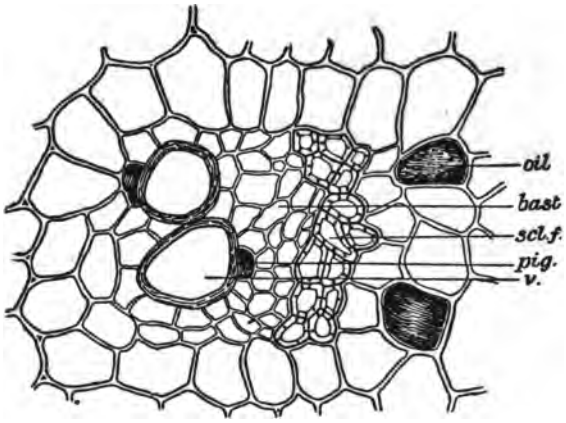


Fig. 49.—Cross-section of Ginger rhizome showing a vascular bundle and parenchyma; oil, oleoresin sacs; scl. f., bast fibres; v, wood vessel (Vogl).

To remove the starch grains which interfere by their great numbers mount a section in chloral hydrate solution and warm gently. The shape of the parenchyma, wood bundles and the secretion sacs can now be seen more easily.

Mount a section in caustic alkali (5 per cent.). The starch dissolves and the secretion sacs turn a deep reddish-brown, because of the union of alkali with the resin. In the liquid of the sacs unchanged oil drops are visible. Use high power also in the above experiments and also study the starch grains carefully.

MUCILAGE SACS IN MARSH-MALLOW ROOT.

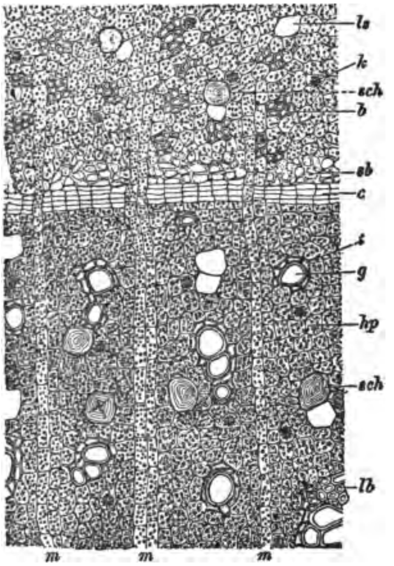


Fig. 50.—Cross-section of Althæa root. sch, mucilage sacs; c, cambium; b, bast fibres; lb, a bundle of wood vessels and fibres; m, medullary rays (Tschirch).

Wrap a piece of the root in wet filter paper until it is soft enough to cut. Make cross-sections and place them in dilute alcohol (not water, because it acts on the mucilage). Mount a section in dilute alcohol or glycerin. Smaller cells densely filled with starch constitute the greatest proportion of the section. Scattered irregularly among these are numerous larger rounded sacs containing a transparent substance — the mucilage sacs.

Add a drop of iodine solution to a fresh section. Most of the cells turn dark (starch), but the mucilage sacs remain as before. In the large central area groups of yellow-stained wood bundles are visible, while in the cortical layer groups of similarly stained bast fibres are seen. A distinct circle of small cells, free from starch, divides the cortical layer from the larger central area. It is the cambium zone.

Remove nearly all of the liquid from the iodine section and add a drop or two of sulphuric acid (diluted with one-fourth water). The mucilage is stained a deep brown and the walls blue (cellulose). Under favorable conditions delicate concentric lines may be seen in the mucilage.

Soften a piece of Elm bark and examine cross-sections in the same manner as in the case of marsh-mallow root. It has large and numerous sacs.

MUCILAGE AND RAPHIDE SACS IN SQUILL BULB.

Crystals are of such general occurrence in widely different orders of the higher plants that there are perhaps none in which they may not be detected. They have been found in nearly all parts of the vegetable structure, more commonly in the interior of parenchyma cells, sometimes in specialized crystal cells.

They occur either singly or in groups. The most common forms are the octahedron and the prism. Sometimes the crystals are much elongated and pointed, like a needle, and are then known as *raphides* (from raphis, a needle). These are generally massed in a compact bundle, like a wheat-sheaf, occupying a large part of the interior of the cell. Raphides are by no means of such general occurrence as ordinary crystals, being restricted to certain orders.

Soak a slice of the scale of a squill bulb in water just long enough to soften it and then place in a mixture of alcohol and glycerin 6-8 hours. Make thin sections and mount in glycerin. The tissue is composed of large, clear, typical, parenchyma cells, with here and there a cell filled with long needle crystals, together with mucilage.

Apply a drop of acetic acid. The needles do not dissolve. To another section add hydrochloric acid; the needles dissolve without effervescence, showing that they are calcium oxalate. Had they been carbonate they would have dissolved in acetic acid with effervescence.

SECRETION RESERVOIRS.

These differ from intercellular air space only in being filled with secretions instead of air. They differ from secretion sacs in that they are spaces surrounded by a number of cells, while the sacs are single cells. The reservoirs are often merely irregular spaces left by the breaking down of one or more cells, but they sometimes have a remarkable regularity of form and clearness of outline.

It has been observed that these spaces are not, as a rule, met with in plants having the simple secretion sacs. The cells which surround the more complete cavities are quite different from the other parenchyma cells, and they are collectively called the *epithelium* of the spaces. These are the secreting cells and the secreted matters are discharged in some manner into the reservoirs, where they accumulate.

Cut cross-sections of a pine needle between pith and mount in water or chloral hydrate solution. Arranged around the section near the periphery are five or six cavities, each having a lining epithelium of flattened, thin-walled cells, and next to these is a circle of glistening, very thick-walled cells (Fig. 65). These structures are channels which carry the turpentine secretion. Also make cross-sections of the stem of a softened clove bud and mount in water. Near the circumference will be found a double row of large cavities, each lined with a membrane of secreting cells. The cavities carry the "oil of cloves."

CHAPTER XXI.

WOOD (LIBRIFORM) FIBRES AND BAST (LIBER) FIBRES.

These fibres belong to the prosenchymatous series of tissues. This series embraces those cells which at maturity lose their nuclei and protoplasm, and, therefore, their distinctively cellular character, and have their walls thickened by secondary deposits. They sometimes contain starch and traces of protein matter, but take no active part in the nutritive processes of the plant. They serve it mainly for strengthening or supporting, and hence have been called mechanical tissues. The elements of these tissues are, for the most part, elongated and oblique-ended or taper-pointed. Among the shorter cells transitions occur. Between them and sclerotic parenchyma, and between the fibrous forms and collenchyma, every gradation may be found.

Bast and wood fibres are found in the so-called fibro-vascular bundles of the plant. These bundles constitute the fibrous framework of the plant, corresponding somewhat to the bony skeleton of the human body. In the leaf they are the system of veins, and in the stem and root the tough resistant portions. Their function is partly to give strength and partly to conduct the fluids of the plant. The cells composing the bundles, therefore, for the most part, have their walls thickened and are elongated in the direction of the length of the organ bearing them. They belong chiefly to the prosenchymatous series, although other tissues are commonly included in the bundles. In some plants, as the stem of the Indian Corn and the petiole of the Plantain, the bundles may be readily separated in the form of tough, stringy masses from the softer surrounding tissue. (Break the petiole of a Plantain leaf and note the tough threads protruding from the broken end. These are the bundles).

Although a number of kinds of tissues are usually found in the fibro-vascular bundles, only two kinds are really essential, namely, *ducts* (and tracheids, which may be regarded as imperfectly-formed ducts) and *sieve* cells. These and their associated tissues always constitute separate longitudinal portions of the bundle. The nature of ducts and sieve cells will be explained later. The portion of the bundle to which the ducts belong is called the *xylem,* which means the *wood,* and that to which the sieve cells belong is called the *phloem* or *bast* (phloem means *bark*). The reason for calling this the bast or bark is the fact that the inner bark of gymnosperms and dicotyledons, also called the bast layer, is composed of the phloem portions of the bundles, which are arranged in a circle. Within these are the xylem portions of the bundles, forming the cylinder of wood.

The term bast was originally given to the inner bark of the Linden tree, which was called Bass-wood tree. It is now applied to the inner layer of any bark. The term liber, which is also given to the inner or bast layer, was applied in a more general way to any smooth, inner bark upon which one could write (liber is the Latin for book).

The peculiar, long, tough, thick-walled cells which impart toughness to the inner bark, making it valuable in the arts, are hence known as bast or liber fibres. It is in the bark of dicotyledons that liber fibres occur most abundantly in the phloem portions of the bundles.

Some plants, for example, the monocotyls, have no true bark, consequently the bast or phloem portions of the bundles do not lie in the inner or bast layer of the bark, and the bast fibres of such bundles are apparently misnamed. However, the term liber or bast fibres has been extended to embrace all those fibres that occur in the phloem portions of the bundles, whether they occur in the inner bark or elsewhere in the plant and whether they occur in gymnosperms, dicotyls or monocotyls.

The term libriform has reference to the general resemblance of the wood fibres to bast or liber fibres. Wood fibres usually differ from liber fibres in being relatively less elongated, less tough and flexible and more strongly lignified at maturity, but there are many exceptions, especially in monocotyls, where the two tissues are often indistinguishable by structure alone. The fibres vary often in length.

Wood and bast fibres may be conveniently studied in a moderately stout Geranium stem, in which they have already been noted superficially in Chapter XI. Make thin cross-sections of a Geranium stem and study without staining and also by staining with phloroglucin and hydrochloric acid (red).

The wood circle lies within the cambium zone and surrounds a central area of parenchyma cells, the pith. It is composed of the xylem portions of numerous bundles which have grown together into a solid ring of woody tissue. The wood fibres, in cross section, are seen to be very thick-walled, compactly arranged, more or less compressed laterally by mutual pressure, so as to appear angular. They lie next to the cambium zone. The cells are separated by a distinct line, the *middle lamella.* The walls are lignified, as shown by the red stain, the middle lamella being deeper red than the rest of the walls. There are no intercellular spaces. The cells are unequal in size and irregularly arranged. There are delicate stratification lines and occasionally pore-canals in the walls, though these are seen with difficulty.

In the wood ring there are other cells besides those just described, with much larger openings, the ducts or tracheids, which will be described a little later. Wood fibres do not occur in all fibro-vascular bundles, but are nearly always present in woody and herbaceous dicotyls.

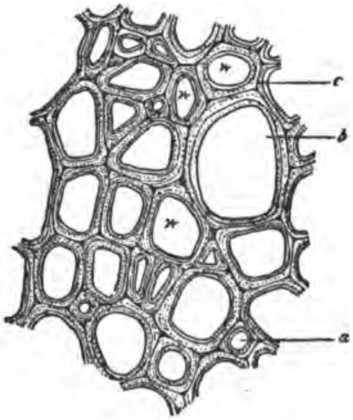


Fig. 51.—Portion of cross-section of wood ring of Geranium stem. w, wood fibres; b, duct; c, middle lamella (Bastin).

Bast Fibres.—Outside the cambium zone is another circle of thick-walled cells, looking in all respects very much like the wood fibres just described. These are the bast or liber fibres. The description of the wood fibres answers also for the bast fibres. With phloroglucin and hydrochloric acid they stain red and the middle lamella deepest. Stratification lines and pore canals can also be seen with care. As a rule bast fibres are not as strongly lignified as wood fibres and take the stains less deeply. In such cases the lignification is chiefly confined to the outer layers of the cell-walls, while the inner layers are more or less cellulose in nature; with double stains, as methyl-green and eosin the outer layers will stain green and the inner ones reddish.

Bast fibres are confined not alone to the phloem or bast portion of fibro-vascular bundles, but are often found in other portions of the plant, for example, the strengthening fibres beneath the epidermis of some leaves; in the ground tissue of many vascular cryptogams. As a rule, bast fibres are thicker-walled than wood fibres, but both kinds may vary considerably in the thickness of the walls, in their lengths as compared with their diameters, in the number of pore canals.

WOOD AND BAST FIBRES IN LONGITUDINAL SECTION.

Treat several longitudinal radial sections of the Geranium stem with Schulze's macerating fluid, as directed under this reagent (see Appendix). Wash them in water and place them in very dilute methyl-green solution for a long time, say 24 hours or more, which will stain only the lignified walls and the cork cells. Mount in water and examine first with low power. On the outer edge is a green layer—the cork cells—scarcely at all affected by the macerating fluid. Next to this is a transparent layer of unstained parenchyma cells more or less injured by the corrosive fluid—the middle bark. Then comes a narrow strand of deeply-stained cells, which on the ends of the section may be seen to be fibrous, and also farther in if the section be not too thick, in which case there may be observed how the fibres are spliced over one another. Next to the bast is another colorless, narrow layer composed of the soft bast and cambium. Adjacent to this is the broader strand of green wood fibres and vessels.

Now tap gently with a teasing needle on the cover glass directly over the sections. The macerating fluid has dissolved the middle lamellas, so that by tapping the cells spread apart and are easily studied individually. The fibres are very long, the wood fibres being often as much as 20 or 30 times as long as wide and the bast fibres even longer; the ends taper to a point, sometimes, however, abruptly, and at times are forked. The walls are smooth, and with careful staining and good light delicate slits are seen running obliquely across the walls. Fig. 52 illustrates both bast and wood fibres in longitudinal section.

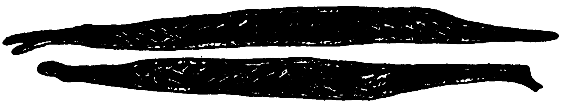


Fig. 52.—Longitudinal view of fibres from stem of Geranium (Bastin).

Sometimes bast and wood fibres are found which are made up of a row of two or three cells and then they have cross-partitions in the lumen or cavity of the fibre.

BAST FIBRES OF CINCHONA BARK.

Bast fibres vary greatly in length and lignification. There are some extremely long, flexible, slightly lignified fibres, for example, Flax, Hemp, Mezereum, on one extreme, and very short, brittle, much lignified fibres on the other extreme, as found in Cinchona bark.

Make cross and longitudinal radial sections of Cinchona bark (calisaya or succirubra) that has been softened in water or alkali.

Examine first a cross-section with low power. In the inner bark there are numerous yellowish, solid-looking quadrangular cells, the cavities of which have been reduced nearly to a point by the great thickening of the cell-walls. These are the bast fibres. They occur singly or in small radial groups of three or more. Incidentally, the student should note carefully the structure of the whole section, as an example of a typical well-developed bark. On the outer edge is a thick layer of small cork cells, deeply colored with natural plant pigment. These have a tendency to flake off in places and are divided here and there by transverse clefts. Next to the cork is the middle bark, consisting of lighter colored, tangentially elongated parenchyma cells. In it is a tangential row of secretion channels. Next to the middle bark is the inner or bast bark, which is divided into compartments by distinct radial lines of cells, two or three rows wide, which are the medullary rays. Between these rays occur the bast fibres and a tangled network of smaller thin-walled cells, consisting of ordinary parenchyma and sieve tubes, the latter not being easily made out.

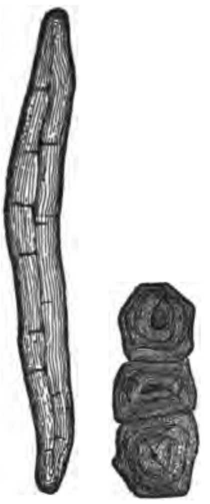


Fig. 53.—Bast fibres of Cinchona calisaya in cross and longitudinal section, showing narrow cavity, pore canals and stratification of walls (reduced from Bastin).

Under high power the excessively thickened walls of the bast fibres are made up of layers which are finely stratified. The walls are also crossed by radiating fine canals (Fig. 53). In a section stained with methyl-green solution the walls are seen to be strongly lignified, but more so in the exterior layers than in those next to the cavity, as is shown by the different depths of color.

Longitudinal Section.—In this the cork and parenchyma look pretty much as in cross-section, but the secretion space, if the section contain one, appears as a long, empty channel, and the medullary rays appear as isolated plates of cells lying across the section. The bast fibres, which are seen best at the ends of the section, are relatively very short, being about six times as long as wide, fusiform or wedge-shaped on the ends. The cavity appears as a line and the stratification is distinct. The fibres are also marked by cross-lines radiating from the cavity (Fig. 53).

Macerate a section in Schulze's solution, as in the case of the Geranium section, wash, mount and separate by tapping on the cover glass. The bast fibres are separated and may be studied more easily. Stain a macerated section in methyl-green, tap out on a slide and note the effect of the stain.

CHAPTER XXII.

TRACHEARY TISSUE.

This includes tracheids and ducts. When speaking of the fibro-vascular bundles, it was said that the xylem portion of the bundle is that which contains the wood fibres and tracheids or ducts. The peculiarity of tracheary cells is that the walls are thickened unevenly, the thick parts being on the inside of the walls and arranged in various forms, giving rise to *spiral, reticulate, scalariform, annular, dotted* ducts, etc.

The cells of tracheary tissue are usually less thick-walled than wood fibres and of larger diameter, and mostly oblique-ended or blunt. They are more widely distributed than wood fibres, as they are found in all vascular plants, i. e., plants containing bundles. (The name fibro-vascular means that there are both fibres and vessels or ducts in the bundles.)

Ducts and tracheids are alike in regard to the markings of the walls. The difference is that a duct is composed of a row of tracheids in which the separating partitions have been absorbed, leaving a duct or vessel. A tracheid is a single cell complete on all sides. The name tracheary signifies the resemblance between some of the ducts and the trachea or wind--pipe of man.

TRACHEARY TISSUE IN GERANIUM STEM.

Make longitudinal radial sections and macerate in Schulze's solution, wash and stain in methyl-green, mount in water. The following ducts may be found in the xylem of the bundles, especially if the cells be separated by tapping upon the cover glass.

Reticulate Ducts.—So called because the thickenings in the cell-wall are in the form of a network, giving a pitted appearance to the wall. The walls are somewhat prismatic and the pits occur on the flat sides. By means of them a lateral osmotic circulation is kept up with other ducts. Notice in some of the ducts, on the oblique ends, large openings where one cell communicates with another. Reticulate ducts are the most numerous in the Geranium, as well as most plants (Fig. 54).

Spiral Ducts.—Widely distributed in plants. The thickening in the wall takes place in a perfect spiral, the part between the turns being very thin and almost invisible and cellulose in nature. There may be two spirals in a duct, one within the other. In other plants ducts with more than two spirals are found. Sometimes the turns of the spirals are connected by cross-thickenings, and then the duct merges into a reticule one. Again, the thickenings may be spiral on one end of the duct, but pass into separate circles on the other, forming an *annular* *duct.* This kind of duct is found associated with the spirals in the Geranium. The two are closely related. The spirals and rings may be close together or wide apart and the rings may be at various inclinations to the length of the duct. The spiral ducts are smaller in diameter than the reticulate (Fig. 54).

SCALARIFORM DUCTS IN FERNS.

These ducts have their thickenings arranged like the rounds of a ladder, hence the name *scalariform.* They occur in many plants, but they are seen to best advantage in the Ferns, where they are beautifully developed and almost to the exclusion of all other woody tissue in the bundles.

Make thin longitudinal radial sections of the rhizome of the official Male Fern or of the Eagle Fern (Pteris aquilina), stain in methyl-green, or phloroglucin and hydrochloric acid, and mount in water. The ducts will be stained green or red; they are mostly prismatic or flat-sided where they press upon one another, and at the edges where the sides meet there is a thickened ridge. Crossing the flat faces are numerous parallel thick ridges separated by very thin places looking like slits. The faces have the appearance of a ladder. The ducts are oblique or taper-pointed, large in diameter and the ends splice over one another (Fig. 54).

Isolate the ducts by maceration in Schulze's solution as in the previous case.

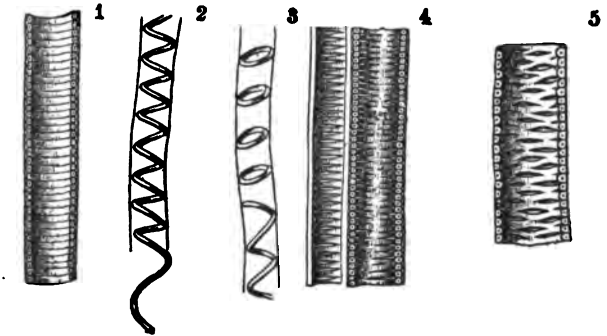


Fig. 54.—Various tracheary ducts. 1 and 2, spiral; 3, combined annular and spiral; 4, scalariform; 5, reticulated.

TRACHEARY TISSUE OF GYMNOSPERMS.

Tracheids With Bordered Pits.—These peculiar cells, although occasionally found in other plants, are characteristic of gymnosperms. In these plants ducts and wood cells are rare, being replaced by tracheids, which constitute nearly the whole of the wood. The tracheids are so peculiar in structure that one may distinguish a gymnosperm from other plants. A few genera of gymnosperms can be recognized by the number and regularity of the markings on the tracheids.

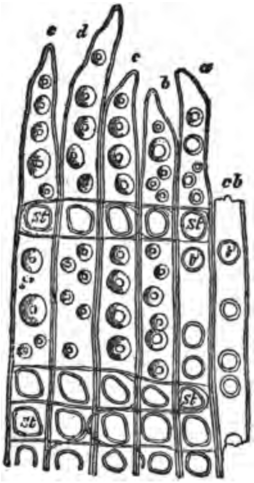


Fig. 55.—Tracheids of pine showing bordered pits, with medullary rays crossing the tracheids (Sachs).

Soften a piece of pine wood by soaking a long time in dilute alkali. After washing thoroughly in water, make longitudinal radial sections by cutting at right angles to the rings of growth. Mount a thin section in water or glycerin. The tracheids are long, tapering fibres, similar to wood fibres, but larger. The surfaces of the cells are marked by a row of pits, each pit being surrounded by a smaller circle inside of a larger one. At times the pits are close together; at other times they are wide apart. Lying across the fibres at intervals are rows or plates of cells of the medullary rays. Try the action of phloroglucin and hydrochloric acid on a section.

Now make longitudinal tangential sections by cutting parallel with the rings of growth. In such a section no pits are found on the faces of the cells, but they may now be seen in section on the edge in the cell-wall. In other words, the peculiar pits occur only on the radial faces of the cell-walls.

Find a pit cut exactly through the middle. It forms a lens-shaped cavity in the wall between two cells, opening into the two cell cavities by circular orifices which in flat view appear as the inner small circle of the pit (see above). Running lengthwise through the pit and closing off the cavities of the two neighboring cells is the middle lamella. Here and there between the tracheids occur rows of two or three rounded cells, which are not to be mistaken for the pits. They are much larger, being the lignified parenchyma cells which form the so-called medullary rays. Observe that some of the medullary rays are much wider than others; in the center of these resin ducts may be seen.

CHAPTER XXIII.

LATEX OR MILK TUBES.

Many plants, when wounded, emit a milky fluid varying in color, copiousness, consistency and chemical composition in different plants. This is called the *latex,* hence the name laticiferous tissue. This tissue differs considerably in different plants and is not confined to any particular region or tissue system, but it is most common and abundant in ordinary parenchyma.

The cells of milk tissue have two modes of origin and are generally distinguished as *latex cells,* and *latex vessels.*

Latex cells consist of long cells of indefinite length running lengthwise of the plant with only a few branches. Each tube with its few branches is believed to be a single cell, arising from a single meristematic cell which is already differentiated in the embryo. As the embryo grows, each of these cells grows and branches, the branches sometimes fusing together and thus forming an intercommunicating system. In cross-section they are distinguished from parenchyma cells by their smaller diameter and by being filled with opaque and densely granular substances. The cell-walls are cellulose.

Latex vessels consist of greatly branching tubes, the branches uniting cross-wise and forming a complex network in the plant. They are formed by cell fusions which take place early in the primary meristematic condition by digestion of separating walls. Latex vessels are distinguished from latex cells only by the method of their development.

Latex is of the nature of a waste or excretory product, although it contains albumin and carbohydrates. It contains resins and gums in solution and oily matters, often alkaloids and organic acids. It coagulates and forms a sticky mass upon exposure to the air. India rubber is an example of such dried latex. Latex varies in color in different plants; it may be white, yellow, orange, etc. In Blooodroot it is reddish.

Stems of plants in which latex tissue is to be studied should be cut into pieces and immediately put into strong alcohol, which coagulates the latex and prevents it from running out of the tubes.

LATEX CELLS.

These may be studied conveniently in Euphorbia and Milkweed plants. Make cross-sections of the stem of one of the Milkweeds, stain in methyl-green and mount in water The milk tubes occur in the pith and bark and are distinguished from the neighboring parenchyma cells by their smaller size and densely granular more deeply-stained contents.

Examine a longitudinal section stained in methyl-green. Lying among the parenchyma cells will be found long tubes filled with dense, granular matter, wavy and with only an occasional branch. Each tube, however long, may be looked upon as a single cell.

Apply chlor-zinc-iodine. The cell-walls stain blue and the contents a brown, showing presence of albuminous matter. Alcannin or cyanin solutions would show the presence of oily or resinous matters. These are held in suspension by emulsion and give the latex the milky appearance.

LATEX VESSELS.

These occur in a number of plants, for example, Dandelion, Chicory, Celandine, Poppy, etc.

Make cross-sections of Dandelion and Chicory roots, stain with hæmatoxylin solution and wash thoroughly in water.

The milk vessels are arranged in small groups, and these form, in the Dandelion, concentric circles throughout the whole bark, which, to the naked eye and under low power, seem complete. Under high power the circles are interrupted here and there. In Chicory the milk tubes are arranged in radiating lines through the bark, and by means of the milk tubes alone Dandelion and Chicory can readily be distinguished under the microscope. The milk tubes stain more deeply than the surrounding parenchyma cells and thus stand out conspicuously.

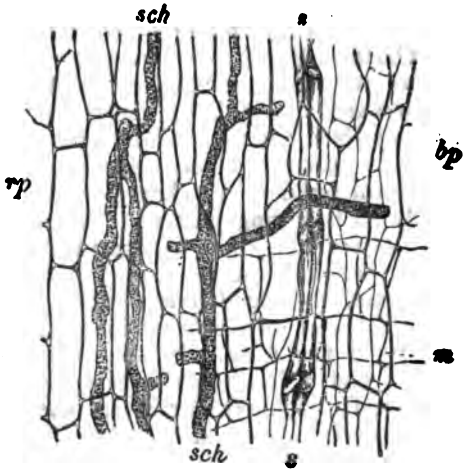


Fig. 56.—Longitudinal radial section of Chicory root showing branching latex tubes, sch (Moeller).

Study a longitudinal radial section of Dandelion and a longitudinal tangential section of Chicory.

The milk vessels form a tangled network in the elongated parenchyma cells of the bark of the root. There are numerous cross-branches connecting the tubes.

CHAPTER XXIV.

FIBRO-VASCULAR BUNDLES AND TYPES OF STEMS.

The nature of a fibro-vascular bundle was considered in the lessons on wood and bast fibres and tracheary tissue. As already stated, although a number of kinds of tissues are usually found in the fibro-vascular bundles, only two kinds are really essential, namely, ducts in the xylem portion, and sieve cells in the phloem portion. The function of the ducts or vessels is to convey the crude sap, that is, the water absorbed by the root hairs, with its dissolved salts; that of the sieve cells is to convey the elaborated sap, that is, the food of the plant manufactured in the leaf. Accompanying the ducts are frequently wood fibres and wood parenchyma, and accompanying the sieve tubes in the phloem are the companion cells whose function is to keep the sieve tubes alive, and phloem parenchyma.

The various types of bundles will be considered now.

According to the relative arrangement of the xylem and phloem masses three kinds of fibro-vascular bundles are distinguished, namely, *collateral, concentric* and *radial.*

Collateral Bundles.—These are characterized by having the xylem and phloem masses lying side by side with the xylem facing towards the pith or center of the stem and the phloem towards the exterior. In the veins of leaves the xylem faces the upper or ventral surface, the phloem the lower or dorsal surface. Collateral bundles are characteristic of the stems and leaves of nearly all flowering plants. They seldom occur in roots. There are two varieties of the collateral type:

The *ordinary* bundle, containing one phloem and one xylem mass, and the *bicollateral* bundle, in which there is one xylem mass between two phloem masses or vice versa. The second variety is found only in the stems of gourd plants (Cucurbitaceæ) and a few others. Some collateral bundles continue to increase in thickness during the life of the plant, and the growing layer is located at the junction of the xylem and phloem, forming a cambium or meristem zone of the bundle. Such bundles are called *open bundles* while those which have no cambium zone, and thus soon cease to grow, are called *closed bundles.*

The open bundles are characteristic of the *stems* of woody dicotyls. An illustration of this kind of bundle has been seen in the Geranium stem. The stems of most monocotyls contain the closed collateral bundles.

Concentric Bundles.—These have a central xylem mass surrounded by a phloem mass or vice versa. There is no cambium zone in this type. The bundle with xylem central is characteristic of nearly all Ferns and Club Mosses. The one with phloem central occurs only in stems and leaves of some monocotyls.

Radial Bundles.—In these the xylem tissues are arranged in radial masses and are separated from one another by the phloem masses, together with some parenchyma cells. Such bundles are characteristic of the roots of all phanerogams and pteridophytes and stems of Lycopodiaceæ.

The following scheme shows the types of bundles and their distribution:

- **Collateral Bundles.** — Stems and leaves of nearly all flowering plants, a few ferns, as the genera Ophioglossum and Osmunda and stems of Equisetaceæ.
  - I. *Ordinary Bundles.* — Having one phloem and one xylem mass.
    - *a. Open Bundles.* —Stems and leaves of woody dicotyls. (Cambium zone present.)
    - *b. Closed Bundles.* — Most monocotyls, the Ferns mentioned and stems of Equisetaceæ. (No cambium zone present.)
  - II. *Bicollateral Bundles.* — Chiefly stems of Cucurbitaceæ (gourd family).
- **Concentric Bundles.**
  - I. *Bundles with xylem central.* Stems and leaves of nearly all ferns and club mosses.
  - II. *Bundles with phloem central.* Stems and leaves of some monocotyls.
- **Radial Bundles.**—Roots of all phanerogams and pteridophytes and stems of Lycopodiaceæ.

COLLATERAL BUNDLES.

Closed Bundles.—Harden a stout piece of the stem of Spiderwort (Tradescantia Virginica) in alcohol and make thin cross-sections. This is a monocotyl. Stain with phloroglucin.

The greater porton of the section is made up of large ordinary parenchyma cells containing starch. Scattered among these cells over the entire section are numerous rounded areas of smaller cells containing no starch. These are the closed collateral bundles. In the xylem, which faces towards the center of the section, will be found from three to five thick-walled ducts, which in many of the bundles are arranged in the form of a V. In some of the bundles the xylem completely surrounds the phloem. On the side of the xylem towards the center of the section there is usually found a large intercellular space. This is often found in closed collateral bundles.

The phloem is composed of small cells, which are mostly sieve cells, accompanied by some parenchyma cells. There is no growing or cambium zone between the phloem and xylem.

The bundles are enclosed by a single row of cells smaller in diameter than the other parenchyma cells of the section and containing little or no starch. This is the *endodermis* or *bundle sheath*. It is poorly developed in this type of bundle and is often not present at all. It will be met with later in perfectly developed form in the concentric and radial bundles.

Note the arrangement of the whole section of this stem. It represents the type of structure of all monocotyl stems. The bundles are scattered without any definite order over the whole cross-section, though they are more numerous in the outer part than towards the center of the section. There is no true bark as exists in stems of dicotyls.

The student should be careful to differentiate between *type* of structure and *details* of structure. Other monocotyl plants have the same plan of arrangement as in the one just studied, but perhaps none looks exactly the same. The case is very much like that of human beings, all of whom are constructed on the same plan, but very rarely do two tally so closely that they cannot be distinguished.

To emphasize the above point, study a cross-section of the young stem of Greenbrier stained with phloroglucin. If the stem be too hard to cut easily, soften it by soaking in dilute alkali (1 or 2 per cent.) sufficiently long. Greenbrier is a monocotyl plant of the harder or more woody kind, while Spiderwort represents the soft, herbaceous plants. In the section will be seen numerous bundles of the closed collateral variety containing, besides vessels, both wood and bast fibres. The bundles are scattered almost over the whole area of the section and are imbedded in a groundwork of parenchyma cells, which, however, have their wall considerably thickened, thus differing from the parenchyma of Spiderwort. The area containing the bundles is sharply divided from a narrow, outer rim of the section, which resembles in a way the bark of a dicotyl plant. Most monocotyl stems have such a more or less distinct dividing line. In some it is a single chain of cells, in others it is strongly developed; it is known as the *cylinder sheath*. In the Greenbrier it consists of numerous hard fibrous cells, which result from the crowding of incomplete bundles in this region.

Note the two very large ducts and several smaller ones in the xylem or wood portion of the bundles. The xylem portion is directed towards the center of the section. In the outer portion next to the two large ducts is an area of softer smaller cells, the phloem, made up of sieve tubes and some parenchyma cells. The whole bundle is enclosed by fibres which, on the inner edge, are called wood fibres, while on the outer edge they are called bast fibres.

Examine also sections of Corn stem, and compare with the above.

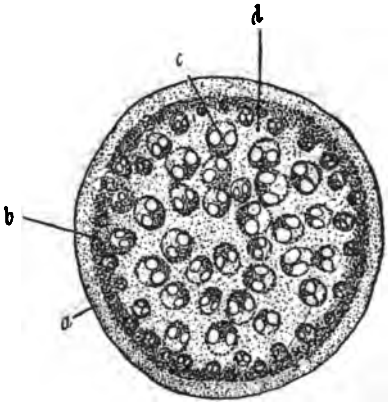


Fig. 57.—Cross-section of stem of Greenbrier. a, cortex; b, cylinder sheath, made up of incomplete bundles; c, a bundle; d, parenchyma cells of ground tissue (Bastin).

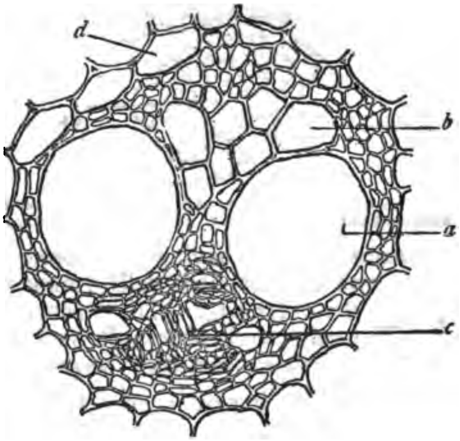


Fig. 58.—Section of a bundle of Greenbrier, magnified. a, large duct in xylem; b, smaller duct; c, phloem mass; d, parenchyma cell (Bastin).

Open Bundles.—These are found in stems of dicotyledonous plants. In herbs they are more or less isolated, and the xylem portions do not form a solid continuous woody cylinder, as in shrubs and trees. The cross-section of the Geranium stem furnished an example of open collateral bundles.

To study the bundles in a woody type of stem, make cross-sections of the stem of Bittersweet and stain with phloroglucin. Surrounding a central area of pith cells will be found a thick ring of cells that stain red, composed mainly of thick-walled wood fibres, which are interspersed with cells of much larger diameter, the ducts. The solid ring of cells is composed of the xylem portions of numerous bundles which have grown together, and the only evidence of their having once been separated is a number of radial rows of elongated cells running through the ring from the pith to the outer edge. These rows of cells are the *medullary rays*. The cells were once soft parnchyma cells between the bundles, but have subsequently become lignified.

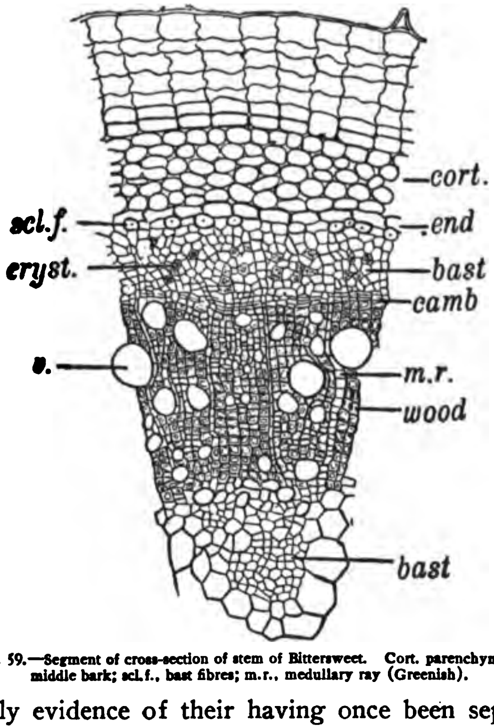


Fig. 59.—Segment of cross-section of stem of Bittersweet. Cort. parenchyma of middle bark; scl.f., bast fibres; m.r., medullary ray (Greenish).

At the outer edge of the ring of wood cells is a narrow zone of small cells, thin-walled, unstained, rectangular in shape and more or less in radial rows. These form the cambium zone or growing layer.

Outside the cambium is a zone of unstained cells composed of sieve tissue and parenchyma cells. This zone is bordered exteriorly by a broken circle of small thick-walled bast fibres. The area between the cambium and bast fibres, including the latter, is composed of the phloem portions of the bundles. The medullary rays are continued through the phloem areas. This region forms the so-called inner or liber bark, spoken of in the lesson on bast and wood fibres. Next to the inner bark is the middle bark, composed of large ordinary parenchyma cells. Beyond this is the outer bark, composed of a layer of cork cells. Only dicotyls have a true bark, and the structure of such a bark is seen in this section. When the bark is peeled off the rupture takes place at the cambium zone, which is soft and easily torn.

In perennial dicotyl stems the cambium zone forms yearly a new layer of woody tissue on its inner edge, thus increasing the diameter of the plant. Moreover, the beginning and end of the year's growth usually differ in appearance, and thus the "rings of growth" are distinguished, and by their number the age of the plant also. Monocotyl stems have no cambium in the bundles after they are mature, hence such stems soon cease to increase in diameter. They are, as a rule, slender stems.

Study the whole cross-section as a type of the more woody dicotyledonous stems and contrast it with the stem of the Spiderwort studied above. It is the definite arrangement of the bundles in a single circle with xylem ends centrally and phloem ends exteriorly, that gives rise to a true bark and a central woody cylinder in dicotyls.

Make a cross-section of a piece of pine wood, showing two or more years of growth. Observe the tracheids of small diameter formed in the fall, and then the sudden transition to tracheids of quite large diameter formed in the spring. Observe also the resin ducts. Study also a cross-section of a Linden stem several years old.

Make cross-sections of stem of Lizard's Tail as a type of herbaceous dicotyledonous plants.

Stain with phloroglucin. The bundles are arranged in a circle, but are not grown together. They are separated by soft parenchyma tissue which forms the greater portion of the section. There is a cambium zone between phloem and xylem. In the xylem, next the cambium, are some large ducts with a few parenchyma cells mixed in. Next to these are some ducts of smaller diameter and then a semicircular zone of thick-walled wood fibres marking the limits of the xylem of the bundle. In the phloem, next to the cambium, is a mass of soft tissue composed of sieve cells, parenchyma cells and a few secretion cells. The phloem is bounded by a layer of thick-walled bast fibres with pore-canals. This layer passes around to meet the layer of wood fibres of the xylem, forming thus a sort of sheath to the bundle. If Lizard's Tail cannot be obtained, red clover stem can take its place. Imbed in celloidin.

Note that the arrangement of the tissues in this stem is the same as that in the Bittersweet, the difference being one of degree rather than kind. There is far less wood in the section. The type of bundles and the arrangement in a single circle is the same.

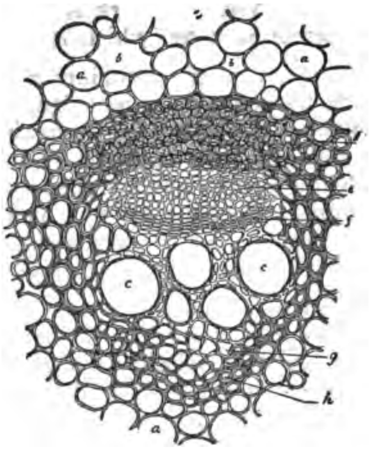


Fig. 60.—Cross-section of a bundle of Lizard's Tail. a, parenchyma; b, air space; c, large duct; d, bast fibres; h, wood fibres; e, soft bast; f, cambium; g, smaller duct (Bastin).

Sketch the whole cross-section of Bittersweet and Lizard's Tail and make a more detailed drawing of a segment of each section.

BI-COLLATERAL BUNDLES.

Make cross-sections of the hardened stem of Pumpkin, Squash or Watermelon.

The bundles consist of a xylem mass between an outer and an inner phloem mass. In the xylem are some very large ducts, looking like large holes, with some smaller ones in the inner portion. There is a large quantity of small-celled parenchyma tissue. The phloem masses consist of large sieve cells and accompanyng parenchyma cells. There are no bast fibres present nor any wood fibres in the xylem. There are two layers of cambium cells, one between the xylem and outer phloem mass, the other between the xylem and inner phloem mass. The rest of the section is made up of very large-celled parenchyma tissue. Examine the sieve tissue for sieve plates, and draw one under high power.

CONCENTRIC BUNDLES.

I. Variety with phloem surrounding xylem.

Make cross-sections of the rhizome of the Eagle Fern (Pteris aquilina) and stain with phloroglucin.

On the exterior is the epidermis brown in color, then several layers of thick-walled fibrous cells, also brown, known as the *hypoderma.* Interior to this is a zone of large ordinary parenchyma cells with starch grains. Then comes a circle of bundles, separated from one another by parenchyma. The bundles may be round or elongated, but never radially. Within this circle of bundles are two elongated masses of thick-walled, fibrous cells, dark colored. Lying between these are two bundles, elongated and larger than those of the circle. The spaces between the objects just described are filled up with parenchyma cells

In the center of the bundles is the xylem composed of large scalariform ducts stained red and a few small parenchyma cells. Surrounding the xylem is the phloem, unstained, consisting of a layer of small parenchyma cells with fine starch grains immediately next to the xylem; then a layer of sieve cells and their companion cells, finally another layer of small starch - bearing parenchyma cells. The bundle is sharply divided off from the surrounding parenchyma by a well-developed ring of elongated prismatic cells, known as the *endodermis* or *bundle sheath.*

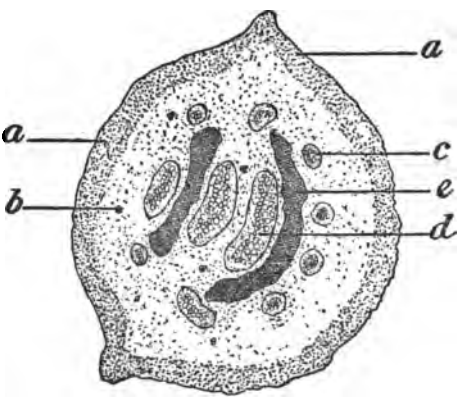


Fig. 61.—Slightly magnified section of stem of Eagle Fern. a, hypoderma. b, small group of dark fibres in cortex; c, one of bundles in a circle; d, one of the two bundles in the center; e, brown mass of fibrous cells (Bastin).

There is no cambium in the bundles, hence Fern stems, just as those of monocotyl plants, do not increase in diameter from year to year. They remain rather slender.

Note the plan of this stem. It is the type on which all Ferns are built. Note also that it differs from the monocotyl and dicotyl types of stem. The two large masses of dark fibrous cells in the central portion of the section are not present in all Ferns.

II. Variety with xylem surrounding phloem.

Make cross-sections of stems of False Solomon's Seal or Sweet Flag, both monocotyl plants, and stain with phloroglucin.

The structure of the bundles is just the reverse of that of the bundles of the fern, in that the xylem cells are on the outside and the phloem is central, but there is no endodermis present. In a few bundles the xylem ring is incomplete and the phloem is continuous with the parenchyma outside the bundle. The concentric bundles, with phloem central, may be regarded as closed collateral bundles in which the xylem has completely grown around the phloem mass.

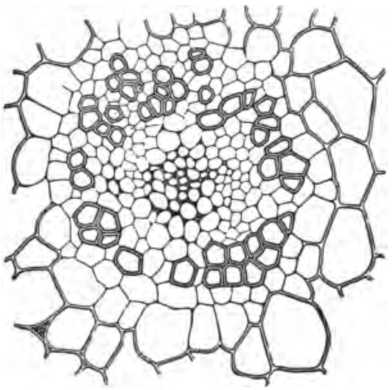


Fig. 62.—Cross-section of a concentric bundle of the rhizome of Sweet Flag, with central phloem surrounded by the xylem vessels (Tschirch).

Both these plants have the arrangement of the parts characteristic of monocotyl stems. In both there is a cylinder sheath dividing an outer border or cortex from the broad central area in which the bundles are scattered. The parenchyma tissue of Sweet Flag (Calamus) has been studied earlier (Chapter XX). The parenchyma of False Solomon's Seal is large, thin-walled, closely packed. Neither section contains cork cells.

RADIAL BUNDLES.

These vary considerably among themselves in regard to the number of xylem rays and their length, the amount of lignification of the cells, the tructure of the pericambium layer and of the endodermis. The number of rays varies from two to forty or fifty in different roots. The number of rays is indicated by the word *arch* with a numeral prefixed. Thus a bundle with two xylem rays is called a *diarch* bundle, one with three a *triarch* bundle, etc. As a rule, dicotyl and gymnosperm roots have fewer rays and a thinner-walled endodermis than roots of monocotyls.

Root of a Dicotyl Plant.—Make cross-sections of the root of the May Apple (Podophyllum peltatum) and stain in phloroglucin. In the center of the section will be found the bundle, which in this plant is usually pentarch, i. e., has five xylem rays. The rays are wedge-shaped, with the broad ends towards the center. At the outer narrow ends of the rays the ducts are smaller in diameter, but are much larger at the broad ends and mostly scalariform. The central portion of the bundle is made up of parenchyma cells, among which may be a few scattered ducts.

The phloem masses lie between the xylem rays, towards their outer ends, and are separated from them by layers of parenchyma cells. The cells of the phloem have glistening walls and may be told by these. The bundle is enclosed by an endodermis of elongated cells, which are thin-walled, as is usual in dicotyl plants. Immediately next to the endodermis are two layers of cells, larger in diameter than the cells of the endodermis or of the phloem and containing some fine-grained starch. These cells are known as the *pericambium* or phloem-sheath. The cells have the power of multiplication and root branches have their origin from them, opposite the xylem rays.

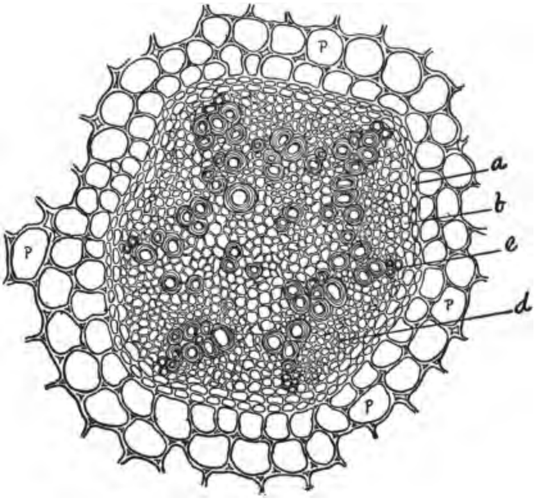


Fig. 63.—Cross-section of root of May Apple, showing radial bundle. a, endodermis; b, pericambium; c, xylem ray; d, phloem; p, parenchyma of cortex surrounding the bundle (reduced, from Bastin).

The significance of the *radial* bundle in roots is that it allows the water absorbed by the root hairs more direct communication with the vessels of the xylem.

The area outside of the bundle is filled with parenchyma cells densely filled with starch grains.

Root of a Monocotyl Plant.—Make sections of the root of Yellow Lady's Slipper (Cypripedium pubescens), a monocotyl plant, and stain with phloroglucin. The xylem rays are about eight in number, longer and better developed than in the previous case. They meet at the center of the section, where there are numerous large ducts and smaller thick-walled cells. The ends of the rays are surrounded by thick-walled narrow cells which reach out to the endodermis, interrupting the pericambium layer in places. The phloem masses lie between the rays; the walls of the cells are thin and glistening. The endodermis is peculiar in that the cells opposite the phloem masses have their radial and inner walls much thickened, giving to these parts the appearance of a crescent, while the outer walls remain thin. The other cells of the endodermis opposite the xylem rays are thin-walled. This is a peculiarity of the endodermis of monocotyl roots. These thin-walled cells allow the water absorbed from the soil access to the water tubes. Make a drawing of the bundle.

Compare with the section of Cypripedium, one from the root of the Corn plant, which is also a monocotyl. There are about fifteen xylem rays, somewhat like those of podophyllum in appearance. They do not reach to the center of the bundle. This is filled up with parenchyma cells, in which there is a circle of 5 very large vessels. Study also sections from the root of the Iris.

The roots of monocotyls undergo very little change as they grow older, but while the *young* roots of dicotyls present the appearance described under the root of Podophyllum, the older ones undergo radical changes and assume the structure of dicotyl stems. In fact, the section of old dicotyl roots looks so much like that of a stem that it is often difficult to distinguish it from a stem section. These changes can easily be followed by making sections of a root at various distances behind the growing point. Study sections of the older roots of a pine and compare with those of the stem of the same age.

CHAPTER XXV.

LEAVES.

The leaf consists of: (1) the fibro-vascular system or framework of veins; (2) the parenchyma or filling; (3) the epidermis, which covers the whole leaf. The parenchyma or mesophyll of the leaf is arranged differently in different leaves, giving rise to two types of leaves, namely, *bifacial* and *centric*.

Bifacial Leaf.—These are always flat leaves, and in section present a distinct upper and lower surface, which are quite different in structure. The parenchyma cells next the upper surface are compactly arranged and elongated perpendicular to the surface. Such cells are known as *palisade* parenchyma. They contain numerous chlorophyll bodies, which give to the upper surface of such leaves the deeper green color, as compared with the lower surface.

The parenchyma next to the lower surface is loosely arranged and scarcely elongated at all and is known as *spongy* parenchyma.

Most any flattened leaf will serve for the study of the bifacial type. An excellent leaf is that of the Rubber Tree (Ficus elastica), because of its toughness and thickness. Leaves bleached in alcohol will be better, as the sections will be more transparent, but best of all will be sections prepared from leaves properly fixed and imbedded in paraffin.

Make sections perpendicular to the lateral veins of the leaf and mount in water or glycerin. Sections of the fresh leaf may be cleared in carbolic acid or chloral-hydrate.

The epidermis on both surfaces is composed of three layers of cells. This is not common to all leaves, but is usually found in tough evergreen leaves. The triple layer affords greater protection, and serves at the same time as a storehouse for reserve water. Here and there along the upper surface, and sometimes on the lower also, occur very large cells with a mass hanging from a stalk attached to the cell-wall, like a bunch of grapes, in the cavity of the cell. The hanging masses are called *cystoliths*. They are not of common occurrence.

Next to the upper epidermis are two layers of elongated cells, the cells of the outer layer being much longer than those of the inner layer, and all are filled with chlorophyll granules. These are the palisade cells.

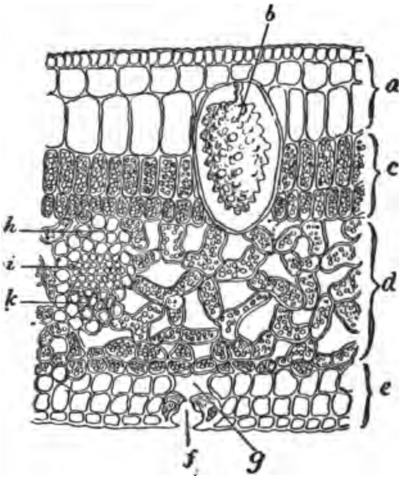


Fig. 64.—Cross-section of portion of leaf of Rubber Tree. a, c, three-layered upper and lower epidermis; c, palisade cells; d, spongy cells; h, xylem; i, soft bast; k, bast fibres, of a small vein; f, stoma; g, air space (reduced, from Rastin).

The rest of the space below the palisade cells is filled in with spongy parenchyma. The cells are not elongated and contain much less chlorophyll than the palisade cells. The cells next the lower epidermis are somewhat compactly arranged.

In the lower epidermis will be found stomata or breathing pores. Some of the pores will be found cut through the middle, giving a clear view of the guard cells. Each pore is seen to lead into a large air space in the leaf, the substomal cavity which is in communication with the intercellular spaces of the leaf. (See Fig. 64.)

The cystoliths consist of a groundwork of cellulose infiltrated with calcium carbonate. On adding a drop of acetic acid to a section the carbonate will dissolve with effervescence, leaving the cellulose mass, which stains blue with chlor-zinc-iodine.

At intervals along the section will be found the collateral bundles of the veins with the xylem always towards the upper side and the phloem towards the lower side of the leaf. The upper and lower faces of a leaf can always be told by noting the position of the xylem and phloem of the bundles of the leaf.

The Work of the Leaf.—The world owes its food supply to green plants which alone are able to manufacture complex substances out of simple inorganic ones, such as carbon dioxide, water and salts. The principal substances manufactured by the plant are starches, proteins and oils, which are stored up as plant foods. The seat of this manufacturing process is the leaf, and the first and most important step is the formation of starch by the chloroplastids in the palisade and spongy parenchyma. The chloroplastids have the power of arresting some of the energy of the sun's rays, and of utilizing it in decomposing carbon dioxide and water, and building up glucose and starch from the products. This activity is due to the chlorophyll (a green pigment) contained in the chloroplastids, and manufactured by the latter with the aid of sunlight. In some manner the physical energy of the sun's rays is transformed into chemical energy as represented in the manufactured chemicals, glucose and starch. The process is called *photosynthesis,* that is, building up with the aid of light.

The source of carbon dioxide is the air, which enters the leaf through the stomata, and permeates it entirely by means of the network of intercellular air spaces.

The carbon dioxide thus comes in intimate contact with the chlorophyll bodies, whose normal position is in close contact with the cell-walls.

Water with mineral salts containing nitrogen, phosphorus and sulphur is brought into the leaf by the veins, which through the medium of the xylem vessels of the stem are in direct communication with the roots. Because of the numerous veinlets, practically few cells of the leaf are farther than two or three cells distant from a veinlet, and therefore from water. Under normal conditions there is an excess of water, which escapes through the stomata into the atmosphere. This latter process is called *transpiration.* Normally then each chlorophyll bearing cell of the leaf is liberally supplied with both carbon dioxide and water.

The precise changes that occur during food manufacture in the leaf are not altogether clear. It is known that carbon dioxide and water are broken down and that oxygen escapes through the stomata; also that starch is found in the chloroplastids. The generally accepted theory is that the chlorophyll, which we may represent by x, first enters into combination with hydrogen of water thus:

$$(1)\ X + H_2O = X.H_2 + O$$

The new compound which has been isolated although it is unstable, combines at once with carbon dioxide, again eliminating oxygen, forming formaldehyde, and x, the chlorophyll is set free to go through the cycle again, somewhat after the fashion of an enzyme or catalytic agent.

$$(2)\ X.H_2 + CO_2 = X + CH_2O + O$$

Whether the formaldehyde condenses directly to glucose or forms intermediate compounds is still not certain. For the present we can represent only the beginning and the end of the change, thus:

$$(3)\ 6\ CH_2O = C_6\ H_{12}\ O_6\ \text{(glucose)}.$$

In most plants glucose is manufactured much more rapidly than it can be disposed of, when photosynthesis is active. It is then that starch begins to form, thus:

$$(4)\ C_6\ H_{12}\ O_6 - H_2O = C_6\ H_{10}\ O_5\ \text{(starch)}.$$

The glucose, as it is manufactured, may be used directly as food, or it may be combined with compounds of nitrogen, sulphur and phosphorus to form protein food. In the latter process, as well as in the formation of starch and oils, the energy required is derived not from the sun's rays, but by the oxidation of some of the foods themselves. In fact all the processes of life are carried on by energy thus set free, and we have an actual reversal of the processes that have taken place in food-making. During the day, therefore, when the sun is shining and photosynthesis is active, oxygen is eliminated so much more rapidly than it is required, that it escapes into the air, but at night, when photosynthesis ceases, carbon dioxide alone escapes into the air.

The food that is manfactured is carried out of the leaves through the sieve tubes in the phloem portions of the vascular bundles, and conducted wherever it may be needed for present use or stored for future use.

Centric Leaf.—This type of leaf is symmetrical, i. e., the structure on one side is the same as on any other side. Palisade cells are not present. Centric leaves are terete, acicular or succulent, and, occasionally, flattened leaves belong to the type.

Most any pine needle will illustrate the type; also leaves of Lady's Slipper, Sweet Flag, Hyacinth, Daffodil.

Make cross-sections of a pine needle by holding it between pith and cutting through the latter. If necessary, clear them in carbolic acid, chloral hydrate or Labarraque's solution.

The leaf is flat on one side, which is the upper or ventral, and nearly semi-circular on the other. The epidermis is a single layer of thick-walled cells which possesses stomata on all sides of the leaf.

Next to the epidermis are two or three layers of thickened fibrous cells, and next to these comes the parenchyma of the leaf, consisting of thin-walled cells, whose walls have been infolded, forming a variety of parenchyma known as *folded.* The cells contain chlorophyll bodies. Arranged at nearly equal intervals in the parenchyma are about five secretion reservoirs, in which the circle of secreting cells is enclosed by one of thick-walled cells.

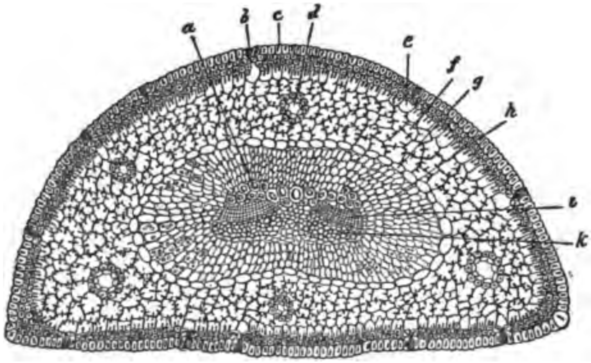


Fig.65.—Cross-section of a pine needle (centric leaf). a, bast fibres of bundle; b, stoma; c, epidermis; d, secretion channel; e, air space; f, folded parenchyma; g, bundle sheath; h, parenchyma; i, phloem, or soft bast of bundle; k, xylem of bundle (reduced, from Bastin).

A bundle sheath separates the central portion from the rest of the section next to which are parenchyma cells surrounding two collateral bundles in the center of the section.

Apply phloroglucin and hydrochloric acid and note result; also iodine solution.

CHAPTER XXVI.

EXAMINATION OF SPUTUM FOR THE BACILLUS OF TUBERCULOSIS, AND OF GONORRHEAL DISCHARGE FOR GONOCOCCUS.

This exercise from the point of view of logical sequence, would have been more appropriately placed in Chapter IV on Bacteria, but from the practical side, it was thought better to take it up at this point, after the student has had more practice in manipulation, staining, and observing under the microscope.

The technique involved is comparatively simple and easily mastered by anyone, with practice. An oil immersion lens is required.

SPUTUM.

Sputum of suspected tuberculosis should be collected in paper cups designed for this purpose, and after it is no longer wanted for use, sputum and cup should be thrown into a furnace and destroyed. Needles, platinum wires or forceps after use should be burned off in a Bunsen flame. Glassware, if contaminated with sputum, should be placed in a closed vessel of water, the water raised to boiling and kept at that point for half an hour. It is then washed with soap and water. It is well, for precaution, that the individual wash his hands in 5% carbolic acid, after working with tubercular sputum, then with soap and water.

If the sputum, which should be of the morning, contains any small yellowish caseous masses, remove one of these with a fine forceps, and spread it into a thin film on a 1-inch square cover-glass. Prepare 3 or 4 such films and set them aside until thoroughly dry.

If the sputum is homogeneous, a small drop of it is spread into a film as just described.

After drying, the films must be fixed to prevent them from washing off. This is done by *flaming* them, that is, each cover-glass with film side up is passed three times through the middle of the upper half of a Bunsen flame. The films must not be overheated, which is avoided by passing the glass horizontally in a circle of about one foot diameter through the flame, and taking about 1 second for the completion of each circle.

After flaming, the films are double stained by the Ziehl-Gabbett method which is most convenient and satisfactory. The cover-glass, film side up, is covered with Ziehl's carbol-fuchsin (see Appendix) and, while held in forceps, is heated above a small flame until steam is given off. The hot stain is allowed to act from 3 to 5 minutes. The film must not become exposed and dry while heating, which is avoided by adding more stain as evaporation takes place. Finally the stain is washed off in water, and the film is covered with Gabbett's methylene blue (see Appendix) for 1 minute. This is then washed off, and if the film is not blue, the stain is again added for a few seconds, until the fuchsin stain has completely disappeared. The film is allowed to dry, and mounted on a slide in a drop of balsam. On the cover-glass is placed a drop of cedar oil and the bacillus is sought for by the immersion lens, with strong illumination and careful focusing.

The fuchsin solution first stains all the bacteria and other cellular constituents of the sputum, but the strongly acid Gabbett solution decolorizes and counterstains everything blue, except the tubercle bacilli, which retain their red fuchsin stain. These bacilli are characterized by the difficulty with which they stain with aniline dyes and by the tenacity with which they retain the dye when once stained.

The tubercle bacilli appear as slender red rods slightly curved, and often lying over on one another, forming a V or an X. They do not occur in chains. The only organism with which they can be confounded are the smegma bacillus and the bacillus of leprosy.

In the examination of urine, some sediment is spread into a film, allowed to dry, flamed and stained as described under Sputum. It is important to keep in mind the smegma bacillus as a source of error. The differentiation is made by decolorizing for 10 minutes with absolute alcohol after staining with carbolfuchsin. The alcohol decolorizes the smegma but not Koch's tubercle bacillus.

GONORRHEAL DISCHARGE.

The same care must be taken to avoid infection as mentioned above under Sputum.

The pathognomonic sign of gonorrheal infection is the presence of the gonococcus. This organism is biscuit shaped and occurs in pairs, the flattened sides lying adjacent. It should be looked for in every case of urethritis and ophthalmia.

A small drop of the pus is spread into a thin film on a glass slide, dried, and flamed as described above under Sputum. Loeffler's solution of methylene blue is then placed on the film and allowed to act for about half a minute, when it is washed off with water. The film is dried, a drop of cedar oil placed on it, and examination is made with the $^1/_{12}$-inch immersion lens. A cover glass is not necessary. The film can, however, be mounted in balsam with a cover-glass and saved.

The gonococci are to be looked for *within* the pus cells. Diplococci lying between the cells are not characteristic or distinctive of gonorrhea. The finding of the germs within the cells is all that is necessary for a diagnosis. In cases of gonorrheal urethritis, after the visible discharge has ceased, small whitish shreds will be found in the urine. They are also found in chronic gonorrhea. When transferred to a slide by means of a pipette, dried, flamed and stained, they will be found to contain numerous pus cells, in which a few gonococci may be found.

APPENDIX.

A.—VARIOUS REAGENTS USED IN THE STUDY OF PLANT TISSUES.

PERMANENT STAINS.

Delafield's Hæmatoxylin.—To 100 cc. of a saturated solution of ammonium alum, add by drops a solution of 1 g. of hæmatoxylin crystals in 6 cc. of absolute alcohol. Expose to air and light for a week and filter. Add 25 cc. of glycerin and 25 cc. of methyl alcohol. Allow to stand until the color is sufficiently dark. Filter and keep in a tightly-stoppered bottle. The solution should stand for several months before using.

Hæmatoxylin is an excellent *nuclear* and *cellulose* stain. It scarcely stains lignified material. Sections that are not apt to plasmolyze are transferred from water directly into the stain, where they remain for several minutes. They are then placed in a dish of acidified 70% alcohol for a minute or less, and washed well in 70% alcohol. Delicate tissues are first passed through graded alcohols before transferring to the stain.

Bismarck Brown.—Make a saturated solution in 70% alcohol. This is a very good *nuclear* stain. Sections are transferred from 50% alcohol into the stain, where they remain from ½ to 1 or 2 minutes. Wash in 50% alcohol. Over-staining does not take place.

Borax Carmine.—Dissolve 4 g. of borax in 100 cc. of distilled water, and then 3 g. of carmine with the aid of gentle heat. Add 100 cc. of 70% alcohol and filter after a day or two.

Carmine is a *protoplasmic* and *nuclear* stain. Sections should be in the stain for about 24 hours, and should then be transferred into acidified 70% alcohol until they become bright and transparent.

Safranin.—Dissolve 1 g. of safranin in 50 cc. of 95% alcohol and add 50 cc. of water. Sections are passed from 50% alcohol into the stain, where they remain for about 24 hours. They are then treated with acidified alcohol, and washed in 70% alcohol. The stain colors *lignified* and *suberized* material.

A good stain to use with safranin is Delafield's hæmatoxylin. Stain sections first with safranin as outlined above, after washing in 50% alcohol, pass them into the hæmatoxylin for 1 to 3 minutes, etc. By this treatment, the cellulose walls are stained violet, those lignified or suberized are stained red.

Fuchsin.—Dissolve 1 g. of fuchsin in 100 cc. of 95% alcohol and add 100 cc. of water. It stains different tissues different shades of red, which persists in *lignified* and *suberized* tissues, but is easily washed out of cellulose walls.

Acid Fuchsin.—Dissolve 1 g. of acid fuchsin in 100 cc. of water. It is excellent for staining *plastids* and *crystalloids*. It acts quite rapidly, generally 1 to 3 minutes being sufficient. The dye is the sodium salt of rosaniline trisulphonate. It is known also as Ruby S.

Iodine Green.—Dissolve 1 g. of iodine green in 100 cc. of water. It stains *lignified* and *cutinized* tissue more rapidly than cellulose. A 2% solution of acetic acid with iodine green dissolved in it, is fine for the instant fixing and staining of nuclei of fresh material.

A good double stain is iodine green and acid fuchsin. Stain in aqueous iodine green for 12 to 24 hours and wash in water until the stain is removed from the cellulose walls (when seen under the microscope). Then stain in acid fuchsin for 2 to 10 minutes. This should stain the cellulose walls but should not act long enough to stain the lignified ones. Pour off the stain and pass at once into 95% alcohol and then into absolute alcohol, not allowing the transfer to take longer than from 10 to 30 seconds in each alcohol. Clear in xylol and mount in balsam.

Methyl Green.—This can be used and acts like iodine green, but the washing must be much more rapid.

Gentian Violet.—Dissolve 1 g. of the dye in 100 cc. of water. Transfer sections from water into the stain, where they may remain from 1 to 30 minutes. Alcohol dissolves out the stain very rapidly, and therefore the sections must be transferred directly into 95% alcohol, and after 2 to 3 seconds into absolute alcohol, where they may remain 5 to 10 seconds. If a differentiation in the stain is desired, use oil of cloves; it is a solvent of gentian violet, but it dissolves the stain more quickly from some structures than from others. After the desired effect has been obtained, replace the oil of cloves by cedar oil, or xylol, and mount in balsam. Gentian violet is the best stain yet discovered for achromatic structures.

Eosin.—Make a 1% solution in alcohol, water or oil of cloves. The last-named solvent is particularly useful as sections are cleared and stained at the same time. One to two minutes is generally long enough for staining. Eosin is a protein stain.

Hæmatoxylin and eosin make a good combination in double staining, using eosin last.

Erythrosin.—This is really an eosin, and now used almost exclusively in plant work in place of eosin. Make a 1% solution in distilled water or in 70% alcohol. It stains rapidly, 30 seconds to 1 to 2 minutes being long enough. When double staining, use it last.

Methylene Blue.—A 1% solution in 95% alcohol is generally used. It is a good nuclear stain, and for studying bacteria.

Haidenhain's Iron Hæmatoxylin.—This is without doubt the best stain for general cytological work. Two solutions are used: (1) a 2.5% aqueous solution of clear amethyst-colored crystals of iron ammonium alum, and (2) a 0.5% aqueous solution of hæmatoxylin, which is best prepared by dissolving the crystals in the least quantity of absolute alcohol and adding this by drops to the required amount of distilled water and shaking after each drop. A few crystals of thymol may be added as a preservative.

The alum solution acts as a mordant, that is, it prepares the tissue for the action of the dye. Sections are placed from water into the alum solution for several hours or over night. They are then washed in water 5 minutes and placed in the hæmatoxylin solution several hours or over night, after which they are washed 5 minutes and again placed in the alum solution. The latter rapidly extracts the stain and the section must be watched under the microscope until the proper degree of differentiation has been secured. The time required for sections stained over night may vary from ½ to 1 or 2 hours. The sections are then washed in water for at least an hour, for if any iron alum remains, they will fade. Dehydrate, clear and mount in balsam.

Picro-Nigrosin Solution.—Add enough of a strong aqueous solution of nigrosin to a saturated solution of picric acid in water to produce a deep olive-green color. This is a good nuclear stain and a good double stain, the nigrosin going to the cellulose and the picric acid to the lignified tissues. In some cases as much as 24 hours may be required for staining.

TEMPORARY STAINS.

Potassium Iodide-Iodine.—Dissolve 1 g. of iodine and 4 g. of potassium iodide in 10 cc. of water, then dilute with 185 g. of water. It is one of the most useful stains. It colors starch blue, protoplasm and protein yellowish-brown, lignified cell-walls deep brown; together with sulphuric acid it stains cellulose blue.

Chlor-Zinc-Iodine.—Dissolve 10 g. of potassium iodide and 0.15 g. of iodine in 10 cc. of water. Add this solution to 100 g. of a solution of zinc chloride of specific gravity 1.8 and mix thoroughly. This reagent may be used either on fresh or alcoholic material, and the specimen on the slide should be nearly dried before applying it. Cellulose is colored (often slowly) blue or violet, lignified walls yellow, cork yellow to brown, protoplasm brown, and starch swells and is colored blue.

Phloroglucin.—A solution of 1 g. in 100 cc. of 90 per cent. alcohol. The solution in time turns dark and should not be kept more than three or four months. The section is first immersed in the reagent, say for five minutes, after which it is nearly dried and a drop of strong hydrochloric acid added to it. Only lignified substance is colored. The color varies from pale to dark red, according to the amount of lignification.

Aniline Sulphate.—This salt is sparingly soluble and a saturated aqueous solution is used. The section is mounted in a drop of the solution and a drop of sulphuric acid is added. Lignified material assumes a yellow color.

Cyanin.—A solution of cyanin in equal parts of alcohol and water is used to test for fats, which are colored a beautiful blue after one-half hour's immersion. Glycerin may be used to wash out the superfluous stain.

Alcannin.—Macerate 20 g. of alcanet root in 100 cc. of 90 per cent. alcohol for a week and filter. Dilute 5 cc. of the tincture with an equal volume of water just before using and immerse the sections for several hours. The reagent is a test for fats, resins and volatile oils, which assume a red color. Cutinized and suberized cells are also stained red, though not so deeply.

Iron Ammonium Alum.—A saturated aqueous solution is used as a test for tannins, which form a bluish-black or greenish-black precipitate. It should be remembered, however, that occasionally other substances, usually related to the tannins, may be present, which are capable of forming dark-colored precipitates with ferric salts.

KILLING AND FIXING REAGENTS.

95% Alcohol.—This may be used for material which is to serve only for rough work. It kills and fixes at the same time, and the material requires no washing afterwards. It, however, causes protoplasm to shrink.

Absolute Alcohol.—This is a fair killing and fixing agent, causes little shrinking of protoplasm, and saves time if material is to be imbedded in paraffin or celloidin. The time required for fixing in either absolute or 95% alcohol is about the same; in the case of the onion root tip, it is about one hour.

Acetic Alcohol.—To counteract the tendency of alcohol to cause shrinkage, a mixture of 2 vols. of absolute alcohol and 1 vol. of glacial acetic acid has been used with success. It fixes in

15 to 30 minutes. After fixing, the acid is washed out by frequent changes of absolute alcohol.

Carnoy's Fluid.—A mixture of 6 vols. of absolute alcohol, 3 vols. of chloroform, and 1 vol. of glacial acetic acid. It penetrates very rapidly. After fixing, the acid must be washed out in absolute alcohol.

Chromic Acid.—A 1% solution in water gives good results. However, it does not penetrate well, therefore the chromic acid is generally combined with other agents, as acetic acid, or acetic and osmic acids.

Chromacetic Acid.—A good formula for general work is this: 0.3 g. chromic acid, 0.7 g. glacial acetic acid, in 100 cc. distilled water.

Flemming's Fluid.—This fixing solution on the whole gives very good results. Have on hand a mixture of 25 cc. of 1% aqueous chromic acid solution, 10 cc. of 1% acetic acid and 55 cc. of distilled water. When wanted for use, add to the mixture 10 cc. of 1% aqueous solution of osmic acid. This fluid is expensive on account of the osmic acid. It is especially recommended for the study of cell division.

All chromic acid killing reagents require from 12 to 24 hours. The material afterwards must be thoroughly washed from 3 to 5 hours in running water. It is then ready to be dehydrated.

Picric Acid.—A saturated solution in water, or 70% alcohol is used. It penetrates well and is to be recommended for all more or less woody tissues. The time required for fixing varies from 3 to 24 hours. Material fixed in picric acid, whether aqueous or alcoholic, should always be washed in 70% or 50% alcohol, as water is injurious. Picric acid can be combined with other fixing reagents.

Corrosive Sublimate.—A saturated aqueous solution is used. It gives best results when combined with either acetic acid or picric acid.

Corrosive Sublimate and Picric Acid.—Prepared by mixing 3 vols. of a saturated solution of corrosive sublimate in 35% alcohol and 1 vol. of a like solution of picric acid. The two solutions are mixed at the time when wanted for use. It is an excellent fixing reagent for all stem material, requiring 6 to 24 hours. Wash in several changes of 35% alcohol and then dehydrate.

Formaldehyde.—A 2 to 4% solution in water is very good for material to be used in rough work. For material like pine wood, aristolochia stem, etc., it can hardly be surpassed.

Fixing Solution for Leaves.—Two stock solutions are kept on hand:—(1) a solution of 2 g. of potassium dichromate in 100 cc. of a 5% solution of acetic acid; (2) a saturated solution of corrosive sublimate in 35% alcohol. For use, mix equal volumes of the two solutions. The time required for fixing is 24 hours, after which the material is washed for 4 to 5 hours in running water.

DEHYDRATING REAGENTS.

Alcohol is used almost exclusively. The following formulas enable one to make other grades from 95% alcohol. The strengths are approximate, as shrinkage of the mixtures is not taken into account.

To make 15% alcohol, mix 15 cc. of 95% alcohol and 80 cc. of water.

To make 35% alcohol, mix 35 cc. of 95% alcohol and 60 cc. of water.

To make 50% alcohol, mix 50 cc. of 95% alcohol and 45 cc. of water.

To make 70% alcohol, mix 70 cc. of 95% alcohol and 25 cc. of water.

To make 85% alcohol, mix 85 cc. of 95% alcohol and 10 cc. of water.

CLEARING REAGENTS.

The following reagents are used to make sections more transparent, so that the parts may be seen better. When such a reagent is used before infiltration of paraffin, its real purpose is to replace the dehydrating reagent with one that is a solvent of paraffin. In the making of permanent mounts, the clearing agent must be a solvent of the mounting medium.

Xylene (sometimes called Xylol).—This is an excellent liquid to precede infiltration with paraffin, also for clearing sections before mounting in balsam. The material must be perfectly dehydrated before placing it into xylene.

Chloroform.—For some purposes, as the infiltration of very small objects, such as root tips, stamens, etc., with paraffin, it may be better than xylene, as it is more easily removed than the xylene. But for clearing sections before mounting in balsam, it is inferior. Its chief use now lies in its power to harden celloidin when specimens are imbedded in the latter.

Oil of Cloves.—This is most excellent for clearing sections before mounting in balsam. It will clear from 95% alcohol, but it is better to clear from absolute alcohol. It cannot be used as an agent to precede infiltration with paraffin. As oil of cloves is a solvent of celloidin, sections imbedded in the latter must not be cleared in it, unless the object is to dissolve the celloidin matrix surrounding the sections.

Cedar Oil.—Some stains, gentian violet for example, are more or less soluble in oil of cloves. In such cases, it is well to use the oil rather carefully, and when the proper effect has been obtained, to replace it with cedar oil before mounting.

Eycleshymer's Clearing Fluid.—A mixture of equal volumes of bergamot oil, cedar oil, and carbolic acid. It clears readily from 95% alcohol, and is therefore very useful in mounting celloidin sections, when it is desirable to preserve the celloidin matrix.

Glycerin.—Pure glycerin clears gradually. It is the clearing agent when sections are mounted in it, or in glycerin-gelatin.

If sections are not to be mounted permanently, the following clearing agents will be found useful, especially if cell walls alone are to be studied without interference by the contents of the cells.

Chloral Hydrate Solution.—Chloral hydrate crystals, 5 g., dissolved in 2 cc. of water. It is a very valuable reagent. It causes shrunken cells to expand, and dissolves starch, proteins, resin, volatile oils, chlorophyll, etc. When saturated with iodine, by keeping a few crystals of the latter in it, it is employed to detect small starch grains.

Potassium Hydroxide Solution.—For general use dissolve 5 g. of the solid hydroxide in 95 cc. of water.

Labarraque's Solution (sodium hypochlorite) is also used as a clearing and bleaching agent, especially for cells rich in protoplasm, for example, meristem cells. Time of action 5 to 15 minutes. It is excellent for bleaching sections, etc., in which the natural plant pigment is too dark to allow a clear view of details. The reagent should not be allowed to act longer than necessary. Prolonged action results in the extraction of lignin from woody tissues, which then give the cellulose reaction with chlor-zinc-iodine.

PERMANENT MOUNTING OR ENCLOSING MEDIA.

Canada Balsam.—This is a thick solution of the resin in benzene, turpentine, chloroform or xylene. If the solution becomes too thick it is diluted with benzene, etc., respectively. The resin is incompletely soluble in absolute alcohol, hence addition of alcohol to the clear solution in benzene, etc., causes a cloudiness. The balsam should be kept in glass-capped, wide-mouthed bottles. Before mounting in balsam sections must be soaked in solutions which are miscible with it. Such solutions are clove oil, turpentine, benzene, chloroform, xylene, creosote, carbolic acid. It would not do, for example, to take a section from alcohol into balsam. Balsam hardens gradually and hence the slide is finished when the cover-glass is placed on. On the whole, Canada balsam in xylene is the best medium for permanent mounting. Anyone wishing to prepare his own balsam, may do so by exposing some of the commercial Canada balsam to gentle heat in a shallow dish, until the residue becomes perfectly hard on cooling. The resin is then dissolved in xylene.

Glycerin.—This is used sometimes as a mounting medium, but it is troublesome on account of the difficulty of enclosing it with cement. Sections must be brought gradually from weaker to stronger grades of glycerin, starting with a 10% solution. Sometimes it is best to place material in 10% glycerin and to allow the solution to concentrate spontaneously.

The following method will be found good for Spirogyra:—Fix in weak chromacetic acid solution for 24 hours; wash in water for 5 to 6 hours; stain in dilute Delafield's hæmatoxylin (1 part of stain to 3 parts of water) for 2 to 3 hours; wash out excess of stain and transfer to 10% glycerin; allow the glycerin to concentrate until it has the consistency of pure glycerin, and then mount in pure glycerin, using only enough to fill out the cover glass.

Glycerin mounts must have the edge of the cover glass sealed, which is done by centering the slide on a turn-table and spinning with a brush a ring of Brunswick black, gold size, or shellac around the edge of the cover-glass. Several coats should be applied, each one after the previous one has dried.

Glycerin-Gelatin.—This is a better medium than glycerin for most objects. It is prepared thus:—7 g. of best French or German gelatin are softened in 42 cc. of distilled water for two hours; 38 cc. of glycerin are then added and the mixture warmed; then 1 g. of pure carbolic acid is added and the whole stirred for 15 minutes. The liquid is now filtered hot through glass wool. It is solid when cold, but melts at 35° to 40° C.

Before mounting in this medium, objects must be gradually brought into pure glycerin. The glycerin-gelatin is then melted in hot water, a drop placed on a warm slide, the object freed from most of the adhering glycerin is placed in it and covered with a warm cover-glass. In cold weather the mount is safe without sealing, but not in warm weather. It is best to seal all glycerin-gelatin mounts as described above.

Carmine-stained sections cannot be mounted in the medium, as carmine is soluble in the gelatin.

Farrant's Medium.—Equal parts by weight of gum acacia; saturated solution of arsenous acid and glycerin. Soak acacia in solution of arsenous acid for several days, then add the glycerin. *Avoid* shaking, which causes air-bubbles. The same method of mounting is employed in this as in the case of glycerin-gelatin. Slides should be finished with a ring of cement.

OTHER MICRO-REAGENTS.

Sulphuric Acid.—Strong acid diluted with one-fourth its bulk of water.

Phenol (Carbolic Acid).—Used as a clearing agent, also for dehydrating specimens when it is not desired to use alcohol. Sections may be mounted directly from this into balsam. Aniline oil may be used in the same way. Aniline is kept free from water by placing in it a stick of solid caustic potash.

Schulze's Maceration Mixture.—One g. of potassium chlorate dissolved in 50 cc. of nitric acid, generally of specific gravity 1.3, but the strength of the acid may be varied to suit the specimen. It is used for the isolation of cells. Sections are placed in the solution and gently heated until the reddish color which first appears in the tissue has disappeared. The whole is then poured into a large quantity of water to stop action and washed well with water. The cells will now be found easy to separate. Sections should not be carried from alcohol to the mixture, but *always from water,* to avoid violent action. Care is needed to stop the action at the right point. The work should be done under a fume-hood.

Ammonio-Copper Hydroxide (Schweitzer's reagent).—This should be freshly prepared when needed by dissolving some precipitated copper carbonate in concentrated ammonia water. The copper carbonate is obtained by adding sodium carbonate solution to a solution of copper sulphate and thoroughly washing and drying the precipitate by exposure to the air. Schweitzer's reagent is a good solvent for cellulose.

Mayer's Albumin Fixative.—50 cc. of white of egg, 50 cc. of glycerin, and 1 g. of sodium salicylate are thoroughly mixed until a uniform liquid results, which is filtered.

Paraffin sections must be firmly fixed to the slide to carry them through the different processes involved in making a permanent mount. For this purpose, a small drop of the fixative is smeared evenly over the surface of a slide, and all excess is wiped off so as to leave a mere film. A portion of the paraffin section-ribbon can now be floated on a drop of water on the slide, which is then warmed *carefully* until the paraffin becomes perfectly smooth. Care must be taken not to melt the paraffin, but to only soften it. The slide is then placed aside until perfectly dry.

Acidified Alcohol.—This is prepared by adding 1 drop of concentrated pure hydrochloric acid to 50 cc. of alcohol. The grade generally used is 70%, although any other grade of alcohol may be used.

For general laboratory practice, it is best to have on hand a stock solution of 1% acid in 70% alcohol. This is diluted to $^1/_{10}$% as required.

Hydrogen Peroxide.—To remove the dark discoloration due to osmic acid when used as a fixing reagent, a mixture of 5 cc. of 3% hydrogen dioxide solution with 95 cc. of 70% alcohol is used.

Hydrofluoric Acid.—A 10% solution of the commercial acid is used for desilification (removing silica).

Ether-Alcohol.—A mixture of equal volumes of ether and absolute alcohol used to dissolve celloidin.

PRESERVING FLUIDS.

When material is to be preserved for future use, care should be exercised in its collection and treatment.

All material must be thoroughly fixed before preserving. One should not attempt to preserve material in too large pieces. After fixing, the fixing reagent must be washed out, and the material is gradually transferred to 70% alcohol, or, in the case of hard stems, into a mixture of alcohol, glycerin and water. A good preservative, which at the same time is a good fixing reagent, is a 2 to 4% aqueous solution of formaldehyde. Sections cut from material preserved in formaldehyde must be washed well to remove all traces of it before they can be stained. Likewise for sections from material preserved in fluids containing glycerin.

B.—SECTION CUTTING AND METHODS FOR MAKING PERMANENT MOUNTS.

SECTION CUTTING.

Only very thin objects are suited for examination under the microscope, and the higher the power the thinner must be the object. It is evident that in order to study large bodies, as the organs of plants, thin slices or "sections" must be made. Such sections should be of as nearly equal thickness in all parts as possible. A *transverse* or *cross* section is one at right angles to the long axis of the object. A *longitudinal* section is one parallel to the long axis of the object. In studying stem and root structure, three sections will be necessary to understand the structure fully. These are the transverse, the longitudinal radial, and the longitudinal tangential section. The *longitudinal radial* section lies in the plane of the radius, in other words, it is parallel to a medullary ray. The *longitudinal tangential* section is parallel to a plane tangent to the cylinder, and is therefore at right angles to the radial section.

Whether sections can be cut without special preparation of the material, depends upon its nature and the question to be solved regarding it. Likewise much depends upon one's adeptness and the nature of the work as to whether the section will be cut free-hand or with a microtome.

Cutting Sections Free-hand.—Good histological work can be done with some material by this method. If the object is large, it is held in the left hand between the thumb and forefinger, the latter being extended slightly, so as to form a rest for the razor-blade, which is held in the right hand. Small objects are held in elder or sunflower pith, which is split longitudinally in halves. The object is left protruding slightly, or both object and pith are cut together.

In most cases it is best, in cutting, to keep the knife-blade wet with alcohol or a mixture of equal parts of alcohol and glycerin. Sections should, immediately after cutting, be transferred—best by means of a camel's-hair brush—to water or alcohol, otherwise air will get into the cells and seriously impair the value of the section for study.

A sharp razor is necessary to cut sections successfully. It must always be keen-edged and should be stropped frequently to keep it thus, and, when necessary, honed. It is impossible to cut a thin section with a dull razor. A razor flat on one side is the best. It should always be cleaned after cutting sections. It should be pushed, rather than drawn, through the object, with an oblique or sliding motion, even and steady and never with a to-and-fro or sawing motion.

Cutting Sections with a Microtome.—The microtome or section-cutter is a machine in which the object is clamped in a jaw, which is raised by an accurate screw, while the razor or section-knife slides through it, giving very even and thin sections. In the simple form of microtome, the knife is held in the hand, but more elaborate forms have devices for carrying the knife, or holding it stationary while the object is made to vibrate back and forth against it. In some forms, the thickness of the section is regulated automatically. A microtome evidently is a great advantage, and for very accurate histological work, it is absolutely indispensable in cutting imbedded objects. If the razor is to be held in the hand, it should be pushed across the object with a long sliding motion from point to heel of the razor; likewise when it is held in a carrier, it should be clamped at an angle to make a long sliding cut. This is the rule in all cases except when objects are imbedded in paraffin, when the razor is set perfectly square, or at a right angle to its line of motion. In this case the sections are, so to speak, chopped off, and their edges adhere, thus forming a ribbon which preserves the sequence of the sections perfectly. The razor must be kept so sharp that it will clip a hair, even if it is necessary to sharpen it after cutting only a few sections. It is kept wet with dilute alcohol or dilute glycerin in all cases except when cutting objects imbedded in paraffin, which are cut dry.

METHODS FOR MAKING PERMANENT MOUNTS.

While it may not be necessary in a great many instances to follow any elaborate method in the preparation of material for the lessons outlined in this book, and we can even get excellent results by making free-hand sections, it is self-evident that with more careful manipulation, the results will approximate more closely to perfection. In some instances, it is imperative to follow a most painstaking technique to get good results. Sections made from dead material will not have exactly the same appearance as those made from the material when living. The differences are noticed not so much in the case of non-living tissues (those that have become hard and woody) as in the case of those that were alive and actively engaged in life processes. To see the cells with their contents just as in life, the material must be suddenly killed. It must not be given a chance to change by slow death, in other words, the tissues must be *fixed*.

Killing and Fixing.—This is done by placing the fresh material in one of the reagents already described (page 122). Usually the same reagent is used for both killing and fixing, although these fluids need not necessarily have both functions. A purely killing reagent merely brings the life processes to a sudden close, while a fixing, also called a hardening, reagent hardens the living contents of the cells so that they retain as nearly as possible the appearance in life. To get good results the fixing fluid should penetrate quickly all parts of the object, and to do so, the material should be cut into small pieces, which in most cases should not be larger than ¼-inch cubes.

After fixing, the fixing fluid, excepting alcohol, must be thoroughly washed out of the object, after which it is ready for dehydrating, if it is to be imbedded. If the nature of the object permits, it can be sectioned immediately after washing out the fixing fluid, and the sections are treated as described in Chapter X. But it is soon learned that results are so much better when objects are imbedded, that the imbedding process is taken as a matter of course. The choice of paraffin or celloidin for imbedding depends entirely upon the nature of the object. Soft cellular tissues with comparatively little lignification, are always imbedded in paraffin, while stem and root structures, more or less hard and woody, are imbedded in celloidin, if they can be at all.

Dehydrating.—This process is the same whatever may be the ultimate disposition of the object. Dehydrating means freeing from water, which is done by the use of alcohol almost exclusively. The replacement of water by alcohol must be gradual, else plasmolysis will take place and the object will be ruined (see Chapter X.). The object is taken from water and placed successively in graded alcohols of the following approximate strengths, 15, 35, 50, 70, 85, 95, and 100 per cent. (see page 124). for making dilutions). Of course, if the object in some previous treatment has been subjected to alcohol of a certain percent., the dehydration is begun by an alcohol of the same strength. The length of time for coarse objects to remain in each grade of alcohol varies with the material. For such as onion root-tips, 1 to 2 hours in each grade suffices, but for larger objects less easily penetrated, 12 to 24 hours are necessary. For thin sections about 5 minutes is sufficient. Every drop of one grade of alcohol should be poured off before pouring the next grade on the object.

After dehydrating, the object is ready for imbedding, which will now be considered.

Imbedding in Paraffin.—The object after dehydrating is first placed into a clearing agent (see page 124). Generally the main purpose of a clearing agent is to make tissues more transparent, but at the same time another important function is to replace alcohol. The latter function is very essential in the case of infiltration of paraffin; hence a clearing agent must be selected which is at the same time a solvent of paraffin, for example, xylene or chloroform. The transfer from absolute alcohol into the clearing agent should be gradual; if into chloroform, it is well to transfer first into a mixture of equal volumes of absolute alcohol and chloroform, and then into pure chloroform; if into xylene, three changes may be found necessary, first into a mixture of 3 volumes of alcohol and 1 volume of xylene, then into a mixture equal volumes of each, next into a mixture of 1 volume of alcohol and 3 volumes of xylene, and finally into pure xylene. The object should remain in each fluid from one to several hours, according to its nature.

The next step, the transfer into paraffin, must also be gradual. The best way is to put a small lump of paraffin into the clearing agent with the object to be infiltrated. It gradually dissolves and enters the object. In 10 to 12 hours, the dish with the clearing agent saturated and some paraffin left in excess, is placed in a paraffin bath in order to get a more saturated solution, and finally to drive off the solvent. The temperature of the bath should be kept about a degree higher than the melting point of the paraffin used for imbedding. In the course of 2 to 4 hours, the object in the dish is in nearly pure melted paraffin. It will be well now to pour the fluid out of the dish and to replace it with pure melted paraffin. After 3 to 4 hours more, the infiltration of the object with paraffin is likely to be complete. The time required for infiltration varies from several to 24 hours or longer, according to the nature of the object. To avoid exposing very delicate objects unnecessarily long to high temperatures, it is sometimes advisable to infiltrate first with a low-melting paraffin (45° C.), then to replace this with a higher melting one, say at 54° C., or when a very hard paraffin is required, with one that melts at 58° C.

When infiltration is complete, imbedding is the next step, which is done in the following manner: A small tray is made by turning up the edges of a piece of paper, and it is placed upon a cold object, like stone or iron. Into the tray the paraffin is poured, and the object, by means of a teasing needle, is located in proper position for sectioning. If the paraffin stiffens too soon, it can be melted by means of a heated needle or wire. Of course, several objects may be imbedded in the same block of paraffin, but they must be placed so that they can readily be cut out with a border of paraffin all around them. When the upper surface of the paraffin has become stiff, which can be hastened by blowing upon it, the tray is plunged under cold water. The more quickly the cooling takes place, the better is the object imbedded. The object, once imbedded, may be sectioned at once or set aside for any length of time.

Sectioning is done in the following manner: The paper tray is torn from the block of paraffin containing the object, and the block is fastened on the surface of the object carrier of the microtome by means of some melted paraffin. The sides of the block are now pared down so that the opposite faces are parallel, and only a narrow layer of paraffin surrounds the object. The object carrier is then adjusted in the microtome so that the edge of the paraffin block is exactly parallel to the edge of the knife when the latter is clamped at right angles to its line of motion. As the sections are cut, they adhere by their edges to form ribbons. Sectioning is done with the knife and paraffin dry, and the sections seldom need to be thinner than 5 to 10 microns.

The sections when cut are ready for the next step, namely, staining, but to handle them, they must be fastened upon a slide. The ribbon of sections is divided into suitable lengths, which with glossy side down are fastened upon a slide by means of Mayer's albumin fixative as described under this agent (see page 127).

The following scheme sums up all the steps described above, as well as those still necessary in order to attain a permanent mount of paraffin sections.

SCHEME FOR MAKING A PERMANENT MOUNT OF PARAFFIN SECTIONS.

This may be applied to the leaf of the Rubber Tree (Ficus elastica), using a young leaf and pieces not more than ½ inch wide.

1. Fix (see fixing agents, page 122).
2. Wash in running water, 3 to 5 hours
3. Dehydrate successively in 15, 35, 50, 70, 85, 95 and 100% alcohol, 6 to 12 hours for each as convenient.
4. Clear in 25, 50, 75 and 100% xylene, 3 to 6 hours in each (see page 131).
5. Infiltrate with paraffin as described above (see page 131).
6. Imbed in paraffin (page 132).
7. Cut sections and fasten on slide.
8. Dissolve paraffin by standing slide up in vessel of xylene, 5 minutes or more.
9. Wash out xylene in 100% alcohol. Then transfer slide into 95% alcohol, 50% alcohol, and water, 5 minutes for each.
10. Put slide into solution of iron ammonium alum, 6 to 24 hours, as convenient.
11. Wash in water, 3 to 5 minutes.
12. Place in 1% aqueous hæmatoxylin, 24 hours.
13. Wash in water, 3 to 5 minutes.
14. Place in iron alum solution again, and watch sections until proper difference of color is secured, ½ to 2 hours.
15. Dehydrate successively in 50, 95 and 100% alcohol, 2 to 3 minutes in each.
16. Clear in xylol, 2 to 3 minutes.
17. Mount in balsam and label.

The above scheme will also do for root-tips, using Flemming's fluid as fixing agent.

Imbedding in Celloidin.—When only one section is needed for each mount, and the sections can not be cut well without imbedding, celloidin is to be recommended. Hard roots and stems, and seeds, which can not be cut at all in paraffin, are cut easily in celloidin. It is a form of nitro-cellulose, and occurs in the market in pieces looking like cartilage. Solutions of varying strengths are made by dissolving it in a mixture of equal volumes of absolute alcohol and ether, the ether-alcohol reagent.

An object for celloidin imbedding is prepared in all respects as for paraffin imbedding, up to the transfer into absolute alcohol. From the latter it is passed into ether-alcohol for 6 to 24 hours as convenient, then into a 2% celloidin (2 g. celloidin in 100 cc. ether-alcohol) for 1 to 2 days, next into a 5% celloidin for 1 to 2 days, finally into a thick 12% celloidin for several days, after which it is ready for imbedding, which is done in the following manner:

A thin film of glycerin is smeared over the surface of a Syracuse watch glass to prevent celloidin from adhering too tightly and into the glass the thick solution of celloidin and the object are poured, taking care that the object is well covered and is properly located for sectioning. As in the case of paraffin, several objects may be imbedded in the same dish. By placing the glass under a bell jar with a small dish of chloroform for 24 hours, the celloidin becomes firm enough to handle. It is removed from the watch-glass and trimmed down so as to leave a sufficient border around the object, and then further hardened in 70% alcohol for 1 or 2 days, in which it may remain indefinitely without change. In the case of hard material, the celloidin may be placed in a mixture of alcohol, glycerin and water.

Sectioning is done as follows: A small block of wood that has been kept in ether-alcohol for thorough soaking, is dipped into the thick 12% celloidin, and the celloidin block containing the object, after being washed off with ether-alcohol, is dipped into 2% celloidin, and the two surfaces are pressed together. By dropping chloroform on the cementing layer, it soon hardens, and the combination is placed in 70% alcohol for 2 or 3 hours, whereby the joint becomes thoroughly hard. The block of wood is now clamped properly in the microtome, the knife is set as obliquely as possible, and sections cut while the knife and object are kept constantly flooded with 70% alcohol. As the sections are cut, they are placed in 70% alcohol.

If the celloidin matrix must be allowed to remain, a good double stain is hæmatoxylin and safranin, since these stains may be almost entirely removed from the celloidin by weakly acidified alcohol; also since celloidin is soluble in 100% alcohol, and in oil of cloves, these must be avoided in making a permanent mount, which is accomplished by transferring from 95% alcohol into Eycleshymer's clearing fluid, neither the alcohol nor the clearing fluid being a solvent of celloidin.

If the celloidin can be removed from the sections without any injury to them because of their nature, it is removed and the sections are treated as any others.

The following scheme sums up the steps involved in making a mount of celloidin sections:

SCHEME FOR MAKING A PERMANENT MOUNT OF CELLOIDIN SECTIONS.

This may be applied to the stem of Geranium or Corn, petiole of Burdock, etc., etc. Pieces half an inch or less in length may be used.

1. Fix in corrosive sublimate and picric acid fixing solution (page 123), 24 hours.
2. Wash well in several changes of 35% alcohol, 6 to 12 hours in each.
3. Dehydrate successively in 50, 70, 85, and 95% alcohol, 24 hours or more in each.
4. Ether-alcohol, 24 hours.
5. 2%, 5%, 12% celloidin, 24 hours or more in each.
6. Imbed in celloidin; sections are stored in 70% alcohol.
7. Safranin, 6 to 24 hours.
8. Acidified 70% alcohol, until stain is removed from cellulose walls, ½ minute or less generally.
9. Wash in 50% alcohol to remove all acid, 5 to 10 minutes.
10. Delafield's hæmatoxylin, 2 to 5 minutes.
11. Wash in water, 5 minutes.
12. Acidified alcohol, few seconds.
13. Dehydrate in 95% alcohol, 2 to 5 minutes.
14. Clear in Eycleshymer's fluid, 2 to 5 minutes (page 125).
15. Mount in balsam and label.

Or, if matrix of celloidin can be removed,

13. Dehydrate in 95 and 100% alcohol, which removes most of the celloidin from the tissues.
14. Clear in oil of cloves, which completely removes the celloidin.
15. Mount in balsam.

Silica and other mineral deposits, which make some objects refractory, are removed by transfer of the objects from 50% alcohol to a 10% solution of commercial hydrofluoric acid for 3 to 4 days, with several changes of acid and occasional shaking. A wide-mouth bottle coated inside with paraffin is used to hold the acid. The objects must be washed thoroughly in running water for 3 to 6 hours, after which they may be dehydrated and imbedded in celloidin.

C.—MORE IMPORTANT TEST REACTIONS OF THE PARTS OF VEGETABLE CELLS. (Bower's Practical Botany).

Cellulose Cell-Walls—

1. Colored faintly yellow by iodine.
2. Swollen and ultimately dissolved by sulphuric acid.
3. Colored blue with iodine and sulphuric acid.
4. Colored blue or violet with chlor-zinc-iodine.
5. Stained by solutions of carmine or hæmatoxylin, by methylene-blue and in various degrees by other aniline colors.

Lignified Cell-Walls—

1. Colored distinctly yellow by iodine and by chlor-zinc-iodine, but in case of bast fibres the tint may vary to sherry-brown or even pink.
2. Colored brown and swollen by iodine and sulphuric acid.
3. Colored bright yellow by acidulated solution of aniline sulphate.
4. Colored red with acid solution of phloroglucin.
5. Stained slightly or not at all by solutions of carmine and hæmatoxylin, but readily by aniline colors.

Cuticularized or Corky Cell-Walls—

1. Yellow by iodine.
2. Yellow or brown by chlor-zinc-iodine.
3. Yellow by strong potash; on gradually warming (without boiling) bright yellow.
4. Resist action of sulphuric acid, retaining their clearly-marked outlines.
5. Are not stained by solutions of carmine or hæmatoxylin, but are colored by aniline stains.

Mucilaginous Walls—Resemble cellulose in many reactions.

1. Swell with water and to a greater extent with potash.
2. Do not stain with iodine.
3. Stain red with Hanstein's aniline-violet, blue with methylene-blue.

Calcium Oxalate—Occurs in cells in form of crystals.

1. Insoluble in acetic acid.
2. Soluble without evolution of gas in nitric or hydrochloric acids.
3. Soluble in sulphuric acid, with formation of fresh crystals of calcium sulphate if only small bulk of fluid be present.
4. Are not stained with iodine.

Calcium Carbonate—Occurs as incrustations or crystals; it is soluble in acetic acid with evolution of gas (CO_2).

Protoplasm or Proteins Generally—

1. Yellow or brown by iodine solutions.

2. Yellow by nitric acid; on addition of potash or ammonia a bright yellow color is produced (xantho-proteic reaction).
3. Swell and lose details of structure on treatment with potash, ammonia or Labarraque's solution.
4. Stain readily with carmine, hæmatoxylin, bright red with Hanstein's aniline-violet.
5. Best stains for nucleus are hæmatoxylin, safranin and methyl-green.

Starch Grains—

1. Blue with solutions of iodine in presence of water.
2. Swell in potash and in water above 65° C.
3. Swell in dilute sulphuric acid.
4. Swell and are colored blue with iodine in chloral hydrate.

Inulin—

1. Soluble, but not readily, in cold water.
2. Precipitated as sphere-crystals by alcohol or glycerin.
3. Not colored by iodine, and soluble in potash.

Fixed Oils—

1. Black with osmic acid.
2. Saponified with potash; soluble in ether.
3. Pink with alcannin solution.

Resin—

1. Soluble in alcohol or ether.
2. Red by alcannin solution and blue by Hanstein's aniline violet.

Tannin—

1. Deep brown by potassium dichromate or chromic acid.
2. Greenish blue by ferric salts.

D.—MORE IMPORTANT STAINS USED IN ANIMAL HISTOLOGY.

The following list of stains is given in the belief that it will prove to be of practical value to progressive pharmacists, who are often called upon for information about stains, and to prepare them.

Aqueous Alum-Hæmatoxylin.—One g. of hæmatoxylin crystals dissolved in 300 cc. of water, and mixed with 100 cc. of a saturated aqueous solution of ammonium alum. It is allowed to ripen as described under Delafield's solution in Section A. It keeps 2 or 3 months.

Bismarck Brown.—Solutions most commonly used are,—(a) 1% aqueous solution; (b) saturated aqueous solution made by boiling (3 to 4%); (c) saturated solution in 40% alcohol (2 to 2.5%).

Bismarck brown, in contrast to other aniline dyes, is compatible with glycerin. It can be fixed in nuclei by acidified alcohol.

Carbol-Fuchsin, Gabbett's, or **Ziehl-Neelson's.**—Dissolve 5 g. of crystallized carbolic acid in 100 cc. of water, and add to this solution 1 g. of fuchsin. When the latter is dissolved, add gradually 10 cc. of alcohol. A good stain for bacteria, and used in combination with the preceding one for tubercle bacilli.

Delafield's Hæmatoxylin.—This is described in Section A.

Eosin.—The free eosin is sparingly soluble in water, but readily in alcohol. The sodium salt of eosin is readily soluble in water, and is generally preferred and used. A saturated solution is usually kept in stock, and diluted as needed. As a general rule, $^1/_{10}$ to ½% solutions are used. Such dilute solutions should contain 25% of alcohol to keep them.

Gabbett's Methylene Blue.—Stir *slowly* 25 cc. of concentrated sulphuric acid (sp. gr. about 1.826) into 75 cc of water, and allow the mixture to cool. Dissolve in it 2 g. of methylene blue. It is used as a decolorizer and counter stain in the study of tubercle bacilli. Stain for about 1 minute or less.

Eosinated Methylene Blue, Jenner's.—Equal volumes of a 1.2 to 1.25% solution of sodium salt of eosin and a 1% aqueous solution of methylene blue are mixed and allowed to stand in a dish for 24 hours. The precipitate of eosinate of methylene blue is washed, filtered, dried, and powdered. It is stable. When wanted for use, a 0.5% soultion in absolute methyl alcohol is made, which keeps indefinitely. For more exact details of manipulation, etc., see *Clinical Diagnosis,* by Chas. E. Simon.

Eosinated Methylene Blue, Hasting's.—A mixture made in the following order: (a) 1000 cc. distilled water; (b) 100 cc. of a 1% solution of sodium salt of eosin; (c) 200 cc. of polychrome methylene blue solution; (d) 70 cc. of a 1% methylene blue solution. A green metallic-looking precipitate is formed, which is filtered, dried, powdered and bottled. For use a 0.25% solution of the powder in absolute methyl alcohol is made. It has a purple plum color.

Solution (c) in the formula is made by boiling 2 g. of methylene blue in a solution of 2 g. of sodium carbonate in 200 cc. of water for about one-half hour. The hot solution is poured off from the residue, made up to 200 cc., and partially neutralized with 6 to 7 cc. of a 12.5% acetic acid. It should be alkaline in action, since a slight excess of acid destroys its properties, which cannot be restored by adding an alkali. See reference in previous solution (Jenner's).

Eosinated Methylene Blue, Wright's.—100 cc. of 1% methyene blue are added to 100 cc. of 1% solution of sodium bicarbonate, and the mixture is steamed in an Arnold steam sterilizer for 1 hour. On cooling, 500 cc. of a 0.1% solution of sodium salt of eosin are stirred in. The resulting precipitate is collected on a filter and allowed to dry. For use, a saturated solution (0.3%) in pure methyl alcohol is made, filtered, and further diluted with one-fourth its volume of methyl alcohol.

Giemsa's Stain.—Prepared by grinding 3 g. of azure II, 0.8 g. of eosin (B. A.) in 250 cc. methyl alcohol (Kahlbaum I), and then adding 250 cc. of pure glycerin.

Gentian Violet (for **Gram's Method**).—Made as wanted by mixing 16 cc. of a saturated solution of the dye in alcohol with 84 cc. of a saturated aqueous solution of aniline. Some precipitation takes place and it is best not to use the solution before 24 hours. It begins to lose its power after about 10 days.

Gram's Iodine Solution.—1 g. iodine, 2 g. potassium iodide in 300 cc. of water.

Loeffler's Methylene Blue.—A mixture of 30 cc. of saturated alcoholic solution of methylene blue, and 100 cc. of 0.01% aqueous solution of caustic potash (1:10,000).

Toison's Fluid (for Dilutions).—It has the following composition:

Distilled water, 160 cc.; glycerin, 30 cc.; sodium sulphate, 8 g.; sodium chloride, 1 g.; methyl-violet, 0.025 g.

Some less frequently used stains are alum carmine, borax carmine, neutral carmine, Goldhorn's polychrome methylene blue, Ehrlich's tri-acid.

Good reference books on stains, etc., are Mallory and Wright's *Pathological Technique,* and Chas. E. Simon's *Clinical Diagnosis.*

Microscopical stains, etc., may be bought of Eimer and Amend, N. Y.; E. Leitz, N. Y.; Arthur H. Thomas Company, Phila. Grübler's stains are usually employed.

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